EXPERIMENTS ON THE CONTROL OF EELWORM (Heterodera marioni) BY MEANS OF OLIGODYNAMIC ACTION OF SILVER IONS

by

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Frontispiece.

Three photographs showing the externally visible effect of silver treatment in tobacco seedbeds heavily infested with Heterodera marioni. Seedlings of "White Stem Orinoco" two months old.

Plots 1 and 4: controls.

Plot 3: "Protargol" 1 unit, NaCN 1 unit, NaCl 3 units.

Plot 8: "Protargol" 3 units, NaCN 3 units, NaCl 6 units.

Plot 15: Silver-zinc cyanide suspension: 2 un., NaCl 2 un.

Plot 19: Silver-zinc cyanide suspension: 3 un., NaCl 3 un.

Plot 21: Silver-zinc cyanide suspension: 3 un., NaCl 9 un.

Plot 27: (Comparison) Liquid carbon bisulphide: 3 ounces.

(see page 102 et seq.)

E X P E R I M E N T S
O N T H E C O N T R O L O F E E L W O R M
(Heterodera marioni)
B Y M E A N S O F O L I G O D Y N A M I C A C T I O N
O F S I L V E R I O N S

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INTRODUCTION The first discovery of the plant-parasitic nematode causing root knot was published in 1855 by M.J. Berkeley. Though no detailed description of the species was given by him, there seems to be no doubt that it corresponds to the parasite described by Carl Müller, in 1884, as Heterodera radicicola (Greeff) Müller*). This name was generally accepted until 1932 when Goodey, clearing up many discrepancies, revised the nomenclature and suggested the name Heterodera marioni (Cornu, 1879) Goodey, 1932, which has now become established.

This parasite not only has a vast geographical distribution (practically all countries in the southern hemisphere (Marcinowski, 1909), whereas in the northern hemisphere it is even found as far north as the Fenno-Scandinavian area, following more or less the 5° C year isotherm as its upper limit; cf. Goffart, 1934 b), but also has a tremendous number of host plants, and year after year new records are added to those already listed **).

Heterodera marioni, therefore, can be considered as an

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*) Unfortunately I had no access to the original papers of Berkeley, Greeff and Müller, but regarding this question we may well rely on the authority of T. Goodey, to whose paper (l.c.) I wish to refer for further details.

**) Through own observations and from widely scattered literature I have well over 1500 different species on record.

international pest and it has proved to be a menace to different crops of great economic importance in various countries. In Southern Africa especially the tobacco growers and potato farmers annually suffer severe financial losses through low-grade crops as a result of root damage caused by this nematode. The urgency in this country of an economical means of control prompted me to investigate into the problem. The results of this research I present in this paper.

At the onset of my work, which I began as entomologist at the Tobacco Research Station, Trelawney, Southern Rhodesia, and subsequently continued in a similar capacity at the Central Tobacco Research Station, Kroondal, near Rustenburg, Transvaal, I became more and more convinced that the - one could say "classical" - means of eelworm control so far practised were very much limited in their effect (see page 37 et seq.) and for that reason I tried to develop an entirely new line in this field of research.

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LIFE HISTORY OF THE PARASITE The first-stage larvae *) are free-living and inhabit the soil. On account of the transparancy and minute size of their body **) they can not be seen in the soil with the naked eye, but in root observation boxes (Dean, 1929; Linford, 1939, 1940, 1942 b) their behaviour (at least under laboratory conditions) can easily be followed under the microscope. For simple laboratory routine cultural media of a transparent agar in Petri-dishes or test tubes, in which seedlings are allowed to develop, are very useful. By means of artificial inoculation (egg masses or free larvae can be used) penetration of the roots by the larvae may be induced and their movements and orientation observed with a low power dissecting microscope. Byars (1914) first introduced this practice, which was later followed by many investigators.

In this infective stage the larvae penetrate the growing tip or meristematic tissues of young rootlets, preferably just behind the root cap (Byars, l.c.; Godfrey & Oliveira, 1932; Christie, 1935, 1936; Linford, 1939) ***), passing as a rule between the cells (Christie, 1936; similar observations were already made by Němec, 1910). Christie, however, mentions occasional exceptions where larvae passed through the interior of the cells (cf. also Linford, 1941 b), but normally their penetration causes only slight injury to the

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*) Strictly speaking this is the second stage, as (according to Nagakura, 1930, and Christie & Cobb, 1941) the first ecdysis occurs inside the egg shell, just before hatching.

**) Measurements given by different authors range from 0.375 - 0.500 x 0.012 - 0.03 mm. External conditions (such as temperature, food, host) are most likely to be the cause of variations (Goffart, 1934 b).

***) Steiner (1934) observed instances of non-terminal entrance of the parasites in Echinochloa crusgalli, a weed of American rice fields.

tissues (Treub, 1887).

The possible role of chemotaxis in detecting and penetrating the roots of their favourite host plants by the terricolous larvae was discussed by Steiner (1925) and later by Linford (1939). The experiments of these authors *) supply substantial evidence of chemotactic attraction. In very strong support of this is the supposed function as chemical sense organs of prominent anatomical structures, known as "amphids", which were described by Cobb (1924). Godfrey (1936), on the other hand, states that, in his observations on the behaviour of Heterodera larvae, "there was not a striking chemotropic **) response of nematodes over a distance as great as 2 inches. The movements of the larvae in soil appeared to be random rather than directional". ***)

The topographical and histological demonstration of larvae in the root tissues of the host plant is largely facilitated by staining as described by Godfrey (1935 b), Goodey (1937) and McBeth et al. (1941). Linford (1937 a, 1941 b), however, developed a technique of direct observation in living tissues submerged in cultural media.

After penetrating the rootlets the larvae generally settle between the endodermis and the pericycle with their head in the plerome (which later becomes the stele or central cylinder) and their body in the periblem (Molliard, 1900; Tischler, 1902; Godfrey, 1931; Godfrey & Oliveira, 1932; Christie, 1935, 1936; and others) ****). Normally they are orientated

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*) Fresh wounds appeared to be specially attractive (Linford, l.c.) which explains the commonly observed mass invasions (cf. Godfrey & Oliveira, 1932). Poole & Schmidt (1927) also noticed that in sweet potatoes the "greatest infection of nematodes occurred on physiologically cracked areas".

**) The use of the word "chemotropic" in this case is wrong - according to modern agreement - since tropisms are the movements of sessile organisms and "taxis" is applied to mobile individuals.

***) See further remarks on page 15 et seq.

****) Linford (1942 a) states, however, that "larvae are sometimes (but exceptionally) found in the stele".

tail downwards (Byars, 1914; Steiner, 1934). Apparently the larvae are unable to turn between the cells and so, in the exceptional cases of non-terminal penetration mentioned above, the orientation is inverse when the larvae migrate downwards in the root.

Linford (1941 b) observed that right from the beginning of their invasion the larvae start feeding. After having secured their permanent site in the root tissues they become immobile and undergo three ecdyses, gradually increasing in size.

The length of the different larval stages and consequently of the whole life cycle *) seems to depend entirely on the temperature reached inside the root as shown by the experiments of Tyler (1933 a, c) and Townsend (1937) (cf. also Godfrey, 1926) **). The minimum time required for the completion of the life cycle of females has been determined to be 25 days at 27° C, increasing to 87 days at 16.5° C (Tyler, 1933 a). Ustinov (1939) found a minimum of only 24 days for the female and 17 days for the male.

The last larval stage of the male is of special interest. The larva stops feeding (Nagakura, 1930; Steiner, l.c.) after having reached a certain size and when the next ecdysis takes place the adult is formed in the cyst-like skin of the fourth stage larva (see foot note, page 3). The full-grown males (measuring 1.0 - 1.5 x 0.03 - 0.04 mm: Nagakura l.c.) generally remain in the roots of their host plant,

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*) Townsend (l.c.) showed that a rise in average temperature of 0.86° C increased the number of generations per year by one. In South Florida, U.S.A., he states, where the mean soil temperature is about 25° C, 10 or 12 generations per year may develop.

**) Tyler (1933 a) and Townsend (l.c.) both find to a large extent a fairly neat obedience of the thermal constant law. The deviations, however, of their empirical data from the theoretical predictions - to my mind - are significant enough to warn strongly against too rigid an application of this simplification (see also Pierce, 1934).

but occasionally they wander out into the soil (Byars, 1914; Nagakura, 1930) and may penetrate a new rootlet in search of a female. The males are usually far in the minority. In fact, parthenogenesis (the probability of which was already suggested by Byars, l.c., and Gabriel, 1926 b) seems to be the normal way of reproduction as long as the parasite lives under optimal or rather favourable conditions (Tyler, 1933 b; cf. also Nagakura, l.c., and Steiner, 1934). With a tendency to adverse conditions in their environment, however, males become more abundant *) and can often be seen in close proximity to the females, suggesting the likelihood of fertilization to take place (Bessey, 1911, and others). Indeed, spermatozoa are said to have been detected in the oviduct of female Heterodera marioni by Atkinson in 1889. Nagakura (l.c.) describes the amoeboid spermatozoa, which even in a saline solution showed active movements, but in the literature there is no unambiguous information as to whether actual copulation has ever been observed, although Nagakura (l.c.) writes: "Nach der Begattung stirbt das Männchen bald und die Leiche kommt in der Nähe des ausgewachsenen Weibchens oder im Eiersack vor". He even remarks: "Die Eizellen werden in der Samentasche befruchtet und mit Schale versehen und wandern in den Uterus hinein".

In this parasite, apparently, the sex is not determined genetically, but possibly by means of s.c. "sex realisators" (in the sense used by F. von Wettstein, 1920; see Hartmann, 1933), the distribution of which might be dependent on external influences **).

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*) Linford (1941 a) confirms this from experiments, stating that "males are more abundant in places of poor nutrition".

**) Steiner (1934) suggests that, since the "metamorphosis" of the male takes place during a period of starvation, food shortage in general might induce the final determination of the male sex. This is in accordance with Nagakura's findings that sex differentiation does not take place before the 4th stage of the larval period.

Modern views on the problem of parthenogenesis are extensively discussed by Whiting (1945) and, though the case of Heterodera marioni is not mentioned, his article offers suggestive information on this interesting question. Unless cytological data are obtained as to the genotypes and the distribution of the chromosomes in male and female, however, classification of Heterodera marioni according to Whiting's system will not be possible.

The males are - except for their size and the development of the sexual organs - much like the larvae in appearance. The females, on the other hand, change considerably after every ecdysis, gradually swelling until they finally obtain the shape of a boiling flask, or rather like an electric bulb deprived of its fitting. Sometimes they appear more like a retort, as they may become distorted by the growing root or through lack of space between the cells. They now measure 1.0 - 1.9 x 0.5 - 0.9 mm. (Nagakura, 1930).

The eggs, averaging 0.092 x 0.032 mm in size, are usually deposited in a jelly mass or ootheca *), which enables them to withstand desiccation for a considerable length of time **). the embryo then remaining dormant.

The total number of eggs laid by a single female is generally estimated from 300 to 500, although Godfrey & Oliveira (1932) state that "many egg masses were counted with between

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*) Steiner (1934) reported on an exceptional case observed in Echinochloa crusgalli where no egg sac was found with the females in that host.
Chitwood (1938) found the jelly mass to be of a protein nature, presumably a mucoid.

**) According to Steiner (1937) 18 months of drought can be survived; Thomson (1928) even claims to have kept viable eggs in dried roots over two years on a shelf over a steam pipe; cf. also Godfrey & Koshino (1933), who showed graphically the extent to which protected eggs are more resistant than free eggs. Eggs in roots, especially in woody roots, are able to suffer still longer periods of aridity.

600 and 800 eggs. As many as 1200 eggs were counted from a mass showing that new eggs were still being deposited at the time the egg mass was removed". Godfrey (1931) counted up to 1300 eggs in one egg mass; Ustinov (1939) states that 1800 eggs were laid by one female and Tyler (1933 b) records 1998 eggs from one female. In 1938 she finds "counts of more than 500 eggs to be common" and even up to 2882 eggs from a single female "which showed no signs of approaching exhaustion" were recorded.

Attention was drawn by Christie (1946) to an interesting host-parasite relationship to the effect that a definitive influence on the egg production of the female parasites is exerted by the host. The wide variation in egg numbers counted from females in different hosts finds ready explanation in his observations (cf. also Smith & Taylor, 1947).

Byars (1914) finds the rate of egg-laying to be one egg every 50 to 60 minutes, which is more or less in accordance with the experiments by Ustinov (l.c.), who found that 1800 eggs were laid in 61 days (averaging one egg per 79 minutes). Also Tyler (1933 a) confirmed these figures in that she roughly computed the output at one egg per hour at 22° C, though at the optimum temperature (27 to 28° C) she came to about 36 eggs per day (averaging one every 40 minutes). Godfrey & Oliveira (1932), however, claim to have found a pronounced optimum in their egg-laying records. Though Tyler (1938) criticises their statements, she could show large variations in the rate of egg-laying on different days, which, she writes, "must be influenced, primarily or secondarily by temperature".

The egg-packages, when expelled closely under the surface of the root, may be liberated into the soil by rupturing of the epidermis. Imbibition of water by the ootheca often causes it to swell to such an extent that the eggs burst out. Especially in warm soils the females seem to favour a superficial position in the roots (Godfrey, 1926, 1931; cf. also

Goffart, 1934 b). They may even emerge from the root tissues (as observed by Steiner, 1927, in Freesia; and by Goffart, l.c., in potatoes and Iris), simulating the sugar beet (potato) eelworm, Heterodera schachtii, which is more a semi-endoparasite.

In decaying roots all the egg masses contained will, of course, be set free. As the larvae hatch in this jelly mass, they will either remain in the roots of the original host plant or migrate into the soil in search of a new rootlet. Indeed, several generations of larvae can often be found in the tissues of one root or tuber *), even in the same gall.

Hatching seems to depend entirely on temperature and humidity, though several authors claimed to be able to induce or accelerate this process by means of root extracts or even synthetic substances (Rensch, 1924, 1925 a, b, c; Molz, 1928, 1929, 1932; Goffart, 1934 a; Smedley, 1936) in Heterodera schachtii. Whatever these "inductors" may be, I could never detect any delayed hatching of Heterodera marioni eggs even in pure distilled water at room temperature **).

There has been much argument about the function of the stylet (or "onchium": Cobb, 1924), a hollow, spear-shaped, chitinous structure inside the buccal cavity of the larva. Its tiny canal leads directly into the oesophagus, as was observed by Nagakura (1930). This stylet can be protruded, which is easily watched under the microscope. Irrespective of its suggestive structure, however, Goodey (1935) stated that "there is no evidence of puncturing the cell wall by the stylet", and Christie (1936) similarly writes that "the head of the parasite lies between the cells and there is no evidence that the stylet pierces adjacent walls".

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*) Burk & Tennyson (1941) found evidence of 3 and 4 generations in sweet potatoes, "each successive generation penetrating deeper into the tissues".

**) See also Linford (1941 c) who observed that hatching increased with increased moisture up to 45 % when it was as rapid as in shallow water.

This problem was definitely solved by Linford (1937 a, b, 1941 b, 1942 a) who made an intensive study of the feeding habits of Heterodera marioni. He proved that the stylet was used for puncturing the cells in the neighbourhood of the head of the parasite *). In living root tissues he observed under the microscope how the nematode swings its head in all directions over wide angles in order to draw food from different cells. By means of excellent photomicrographs he shows the stylet inside the nutrient cells and how the saliva (from two well developed salivary glands) is poured out. Apparently pre-digestion occurs externally, the liquefied food being subsequently sucked up through the stylet by means of a pumping action of the oesophageal bulbus. The stylet, according to Linford, is also used for opening a passage way in the root when the larva enters a root and migrates to its permanent location.

An interesting discovery was made by Smith & Quirk (1926) who showed that the parasite inside the root is able to adjust the pH of the host tissues to suit its needs, viz. by making an environment that is too acid more alkaline. The tremendous adaptability of Heterodera marioni, which strikes one on inspection of the list of its host plants, may find some explanation in this observation.

ROOT DAMAGE As a result of the continual puncturing of the cell walls and probably under the influence of the parasite's saliva (Christie, 1935, 1936; Linford, 1937 a, 1941 a; cf. also Goodey, 1935) the cells on which it feeds become quite abnormal. In the plerome coalescence of different

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*) Linford (1942 a) watched one larva continuously for 36 minutes as it thrust its stylet very rapidly at approximately the following rates (expressed in numbers per 30 seconds): 52 while the larva slowly moved its head over the cells; 81 with the head held securely in one place; 58 while again exploring and 107 immediately before puncturing a cell. He further states: "By thrusting at such rates and sometimes for many minutes against a small area of a single cell, the nematode gradually weakens ~~the~~ the resistance of the wall".

cells and abnormal divisions take place. The nematodes may break into such cells (Linford, 1941 b), but this must be considered as exceptional (Christie, 1936) *). Usually the histological changes give rise to the formation of "giant cells", which now serve the parasite as a permanent source of nutrition. These giant cells, already described by earlier authors (Treub, 1887; Vuillemin & Legrain. 1894; Molliard, 1900: "cellules nourricières"; Tischler, 1902; and especially Němec, 1910: "nectarial cells") and more recently in great detail by Christie (1935, 1936) are mainly formed in the vicinity of the phloem vessels **) and generally cause typical swellings (galls) in parasitized roots ***); hence the name "root knot" or "root gall" for the disease.

Galls are usually seen on all roots, especially on the younger and soft ones, but an interesting exception was reported by Nattrass (1940) who observed that infection of the oyster nut (Telfairia pedata) in Kenya is restricted to a single large gall on the main root just below soil level.

In response to the parasite's disturbances, increased division is induced in the pericycle which results in the formation of lateral roots (Molliard, l.c.; Warburton, 1902;

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*) In connection with mechanical injury Linford (1941 a) makes the following interesting remark: "Although intercellular migration of these larvae through meristematic tissue appears to kill very few cells, the mere separation of cells probably would suffice to disturb the coordination of tissue differentiation".

**) Much of the cell material from which the giant cells originate would normally have contributed to the formation of these vessels (Christie, 1935), so that the roots, already at an early stage, are deprived of some most vital organs.

***) No gall formation occurs in Cyclamen and Freesia (Steiner, 1927) and parasitized strawberry plants and Iris show little or no swellings on their roots (Tyler, 1933 c). Such cases, however, are exceptional, at least in ordinary roots, though tubers of various plants (potatoes, Dahlia's, etc.) very often lack external swellings that betray the internal infestation.
On several plants (e.g. Zea mais) the galls, though present, are often very inconspicuous and may easily be overlooked by an untrained observer.

Christie, 1935, 1936; and others), usually just above the gall. It is mainly these roots that enable the host plant to keep alive, since the disorders in the vascular system caused by the giant cells hamper the normal flow of sap. In cases of severe soil infestation, however, these newly formed rootlets are immediately invaded again (Warburton, 1902), so that their benefit to the plant becomes rather problematic. Indeed, as a result, many annual herbs succumb and die off. Perennials and especially plants with lignified roots suffer less, but the influence of parasitism will nearly always be conspicuous. The most striking symptoms in the overground parts of the plant are: lack of growth, "poor stand", yellowish colour and drooping of the leaves with a general tendency to wilt.

Consequent to the above primary injuries are remarkable changes in plant-food intake by the host plant, as was shown by Magistad & Oliveira (1934). Infected pineapple plants absorbed 40 - 50 % less nitrogen than clean plants and while the application of fertilizer increased the nitrogen content of the plant, it did not increase the growth of parasitized plants. In addition to this the average total length and the number of roots per plant were definitely much reduced. Collins & Hagan (1932), to the contrary, did not find evidence of the root numbers being decreased by the presence of the nematodes.

Farris & Jehle (1943) found infested plants deficient in phosphorus though this element was present in the soil and normal plants were also not deficient in it.

SECONDARY INFECTIONS Attendant detrimental effects often occur in that lesions in the root tissues, caused by the parasites, offer easy access to terricolous pathogenic fungi and bacteria (Linford, 1942 a), saprophytic Protozoa, Nematoda, Tardigrada, Acarina, insect larvae, etc. Secondary infections of this kind are common in cotton (Fusarium vasinfectum: Orton, 1912: Miles, 1939; Taylor et al., 1939, 1940; Neal, 1940; Smith, 1941; Smith & Taylor, 1941),

tobacco (Rhizoctonia bataticola: Small, 1928), tomatoes (Fusarium lycopersici: Young, 1939 b; Harrison & Young, 1941; Linford, 1942 a; and Colletotrichum tabificum: Miles & Turner, 1928), Antirrhinum, Carnations, Silky Oak, Rose Apple and Loquat (Fusarium: Small, 1922), cowpeas (Fusarium: Smith, 1941) and is likely to occur in other crops as well.

INFECTION OF OVERGROUND PARTS The common restriction of Heterodera marioni infestations to underground structures of the host plants is obligatory rather than preferential. Numerous instances are known of larvae that some way or other got access to tissues other than roots and seemed to thrive equally well in these unusual surroundings. Gabriel (1926 a) noticed "chez de nombreuses Cucurbitacées des galles de racines produites par Heterodera radicicola Greeff non seulement sur les racines, mais encore sur l'axe hypocotylé", and "...au voisinage de l'insertion des racines latérales".

Steiner et al. (1934) record the occurrence of galls 18 inches to two feet in diameter on the basal stems of Thunbergia laurifolia *) and stem galls above the surface of the soil in Rheum rhaponticum and Begonia. In rice, nematodes were found in the sheath and crown tissues, the subcoronal internode and in the coleptile by Tullis (1934). Cotyledons, stem and leaves of beans germinating in heavily infested soil were severely attacked, causing death of the seedlings, as reported by Steiner (1940) (cf. also Taylor, Proc. of the Root-knot Nemat. Conf., 1938). Sveshnikova (1940) found the nematodes in the stem of Areca rubra seedlings, and "galls on the hypocotyl of cowpea (Vigna sinensis Endl.) and okra (Hibiscus (Abelmoschus) esculentus L.) and both hypocotyl and cotyledons of tomato (Lycopersicum esculentum Mill.) resulting from entry

*) Goodey (1935) states with reference to these enormous galls: "It seems possible ... that these are cases of the coparasitism of Heterodera marioni and the "crown gall" organism, Bacterium tumefaciens". This, therefore, might be another instance of secondary infestation.

of nematodes during germination" are recorded by Linford (1941 a).

By means of some beautiful experiments, Linford (1939, 1941 a) was able to show that practically all tissues appear to be equally attractiv. to Heterodera marioni, provided that the texture of the epidermis permits easy penetration of the infective larval form. Larvae migrated, settled and matured in leaves, stems, flowers, etc. and showed no signs of not feeling at home. Linford (1941 a) concludes that "the demonstrated ability of the root-knot nematode to develop to maturity in leaves and stems indicates that some factors other than nutritional adaptation normally limit this root pathogen to subterranean parts. The problem appears to be one of inability of the larvae to gain access to aerial parts. The extreme sensitivity of Heterodera larvae to desiccation and to irradiation (cf. Godfrey & H nino, 1933; H.) appears most important, for it limits periods of larval migration over exposed plant surfac. to periods of continuous wetness. In contrast, shoot-infesting nematode species generally develop stages tolerant of desiccation. --- It is interesting to note that parasites of roots as a group are not nearly so slender as are several of the species most successful as parasites of aerial parts".

A very striking anomaly was published in 1894 by Vuillemin & Legrain, who observed a case of apparently true symbiosis between Heterodera marioni and Beta vulgaris, Apium graveolens, Solanum melongena, Lycopersicum esculentum, Allium Cepa, Daucus Carota and Brassica Rapa in the dry soils of some oases in the Sahara. The walls of the giant cells seem to lignify to form large cavities that serve as water reservoirs for the plants during the heat of the day. The wall of these cavities, they write, "est fenetrée d'un grand nombre de punctuations, qui permettent aux utricules de puiser l'eau aux vaisseaux et de la céder aux cellules altérées du voisinage.

--- A coté des êtres généralement inoffensifs, qui se démasquent, dans certaines circonstances, comme de redoutables agents pathogènes, cet exemple nous permet de placer une espèce qui, suivant les conditions du milieu dans lequel elle vit, exerce les plus graves conséquences du parasitisme, ou produit les effets les plus salutaires de la symbiose".

BIONOMICS OF THE NON-PARASITIC PHASE Though the free-living stage - especially from the point of view of controlling the nematode - is of much more importance than the parasitic phase, one finds, strangely enough, that very little is known about the behaviour of Heterodera marioni in soil, and little or no experiments have been carried out to solve the various problems that obscure a proper insight of the most efficient way of attack on this worm during a period of exposure in which it is extremely vulnerable. Granted that the larva, under natural field conditions - practically speaking - disappears in the soil, thus evading direct observation, this does not necessarily imply its complete escape from all means of experimentation.

The active movements of the larvae in the soil have always been subject of much speculation. Though various authors have given different estimates of possible distances of migration in horizontal and vertical direction *), no experimental work has ever been published in support of any of these individual opinions. In this connection the question arises as to whether the movements of the larvae are random or directed by any stimulus, physical or chemical. Experiments of Steiner (1925) and Linford (1939, 1941 a) definitely indicate attraction by chemical substances, whereas Godfrey (1936) found that no chemo-

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*) Watson & Goff (1937) estimate the migratory movements to be limited to about a foot per month (cf. also Taylor & McBeth, 1941 b). This figure seems to be generally adopted, however, without experimental proof.

tactic response could be noted (see page 4). However, the one does not exclude the other. Most likely the movements are random when definite chemical stimuli are absent or too weak to enable the larvae to detect differences in concentration. But whenever a larva will come into close contact with the roots of a host plant it might be chemically attracted over a short distance. The degree of chemotactic response, then, may be expected to be proportional to the possibilities of migration, as otherwise too many larvae would die of exhaustion before having reached a host. Moreover, it should always be borne in mind that under natural conditions in the soil - if chemical stimuli would at all be perceivable over longer distances - such stimuli would come from all directions, thus mutually cancelling out their individual effects partially or wholly, which again would result in random movement. Too much stress, therefore, cannot be laid on chemotaxis over longer distances, which then also excludes any directed migration. Random movement, on the other hand, should gradually end in a homogeneous distribution of the eelworms through the soil, in a way comparable with a process of diffusion according to the law of entropy. This would seem to be the most favourable distribution for the species, giving maximum likelihood of "success". <u>But such homogeneity in distribution has never been observed</u>! The nematodes appear to be concentrated in certain foci of heavy infestation with larger areas of thinner population in between. These foci originate from infested plants, where larvae are continuously liberated from the roots, or oothecae containing dormant eggs that start hatching under favourable conditions.

If random movements are indeed prevailing, then the fact that a homogeneous distribution is never realized would suggest that migrations are very much limited. The results of my experiments described in this paper (see page 53 et seq.) seem to support this view.

Temperature and humidity (two factors with seasonal variations) are believed to have special influence on the vertical migrations of the worms (Godfrey, 1924). The point in question - to my mind - is more whether these factors have any effect on the distribution of larvae in the soil rather than on their migratory movements. With a view to the above considerations that the nematodes would only be able to travel over shorter distances, it is obvious that in the top layers of soil many nematodes will be "caught" by quick changes in temperature and moisture that have been proved to be lethal. The result will be a thinning out of the population in these strata *) which could make the impression of the larvae having migrated to greater depths where they would be safe.

Confusing these two principles (viz. migration and distribution) will lead to erroneous conclusions.

Summarizing I tentatively would suggest the following hypothesis. As a result of random movements of the larvae there is a general tendency to homogeneous distribution **) radiating from a focus of infestation, occasionally interrupted by chemotactic attraction when the larvae come into close proximity of a favourable root, limited by the span of life of the nematodes and by adverse factors in the "Umwelt" as may be caused by extreme conditions of temperature and moisture, and further by the soil resistance, oxygen supply and the biotic resistance ***), etc. As an apparently necessary means of counteracting these limiting factors might be considered the tremendous numbers in which this parasite occurs (see page 20) as well as the vast range of its host plants.

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*) Godfrey (l.c.) concludes from experiments that "in winter months a considerable reduction in the total nematode content of the soil appears to be evident".

**) This could be called the "distribution pressure" of the species.

***) See under "Biological Control", page 35 et seq.

The effects of temperature on the activities of the larvae in the soil were studied by Godfrey (1926), Jones (1932) and Tyler (1933 a). Godfrey found 13° C the approximate minimum temperature at which extensive infection will take place (rare infections occurring at 10 and 12° C; Tyler also observed root penetrations at 12° c).

Godfrey (l.c.), Jones (l.c.), Godfrey & Hoshino (1933) and Godfrey et al. (1933) investigated the humidity factor with respect to larval activities and found 100 % moisture to be optimal which proves that water is a natural habitat for the larvae. Lower percentages of humidity were shown to be definitely lethal, depending on the time of exposure. The logarithm of the percentage survival, according to these authors, showed a rectilinear relation with the exposure time (cf. Bigelow, 1921), the slope of the regression line becoming steeper with increasing saturation deficiency. Similar curves were found for the eggs of Heterodera marioni, except that the rate of killing for eggs is much slower than for larvae.

Linford (1941 c) sowed tomatoes and cowpeas in infested soil and found that, when the moisture content of the soil was nearly low enough to cause wilting of the host plants, few larvae entered the roots. He observed that infestation and injury increased greatly when the moisture increased slightly above the moisture equivalent of the soil and decreased in wetter soil. The latter observation is in agreement with the general experience that infestation is always lower in soil soaked with water, especially in waterlogged areas in the field. This, at first sight, seems to be contradictory to the finding that water is a natural habitat for Heterodera marioni larvae, but may find an explanation in oxygen deficiency in such areas, since both the eggs and larvae of this parasite are very susceptible to such a condition. This I was often able to observe in the laboratory when eggs and larvae were confined into small, closed

Table I

Experiment showing the effect of fertilizer on eelworm infestation in Virginian tobacco. Spot treatment, equal to 200 lbs of bloodmeal per acre, applied in alternate rows on 10 plots, planted 3 x 3 ft; $x_{1,i}$ treated, $x_{2,i}$ untreated lots.

Percentages ($p = 100^n/N$) were converted to degrees ($p = \sin^2\varphi$) in order to obtain an estimate of the standard deviation (s) independent of the mean ($\bar{X}$) *).

$x_{1,i}$	N	n	p = %	degr.	$x_{2,i}$	N	n	p = %	degr.
$x_{1,1}$	60	0	0.00	0.00	$x_{2,1}$	65	5	7.69	16.10
$x_{1,2}$	40	2	5.00	12.92	$x_{2,2}$	46	16	34.78	36.14
$x_{1,3}$	67	2	2.99	9.96	$x_{2,3}$	109	30	27.52	31.64
$x_{1,4}$	17	0	0.00	0.00	$x_{2,4}$	53	6	11.32	19.66
$x_{1,5}$	77	7	9.09	17.55	$x_{2,5}$	84	29	34.52	35.98
$x_{1,6}$	31	2	6.45	14.71	$x_{2,6}$	50	4	8.00	16.43
$x_{1,7}$	56	3	5.36	13.39	$x_{2,7}$	95	31	32.63	34.84
$x_{1,8}$	47	3	6.38	14.63	$x_{2,8}$	69	32	46.38	42.93
$x_{1,9}$	42	1	2.38	8.87	$x_{2,9}$	71	11	15.49	23.18
$x_{1,10}$	76	5	6.58	14.87	$x_{2,10}$	96	14	14.58	22.44
total	513	25		106.90		738	178		279.34
mean			3.45	10.69				21.94	27.93

The variances were: $s_1^2 = 37.87°$ Total sum of squares:

$$s_2^2 = 90.22° \qquad s(x_i - \bar{x}_i)^2 = 2639.66$$

variance = 138.93

and the estimated standard deviations:

$$s_1 = 6.15° = 1.15\ \%$$

$$s_2 = 9.50° = 2.72\ \%$$

Analysis of variance (according to Saunders, 1939; for modifications see Appendix):

source of variation	sum of squares	d.f.	variance	F	P
treatments	$rs(\bar{x}_t - \bar{x}_i)^2 =$ 1486.00	1	1486.00	47.23	<0.001
replications	$ts(\bar{x}_r - \bar{x}_i)^2 =$ 870.54	9	96.73	3.07	
error	283.12	9	31.46		

*) see Bliss (1937, 1938); tables in Fisher & Yates (1943) and more detailed in Snedecor (1946).

containers. This susceptibility to oxygen deficiency was also mentioned by Godfrey (1926, 1931). Godfrey et al. (1933) shared the views of Brown (1933) saying that "nematode larvae are not drowned by submergence in water but supposedly succumb to exhaustion induced by their own activity which is increased in water".

The pH of the soil, as determined by Godfrey & Hagan (1933), seems to be of little influence on the activities of Heterodera marioni, "although a slight reduction in degree of infestation of the host plants appeared at pH values of 7.6 - 8.0 as compared with lower values". However, Anagnostopoulos (see Proc. of the Root-knot Nemat. Conf., [illegible] describe" absence of infestation to prevailing pH values [illegible] and above.

A striking effect on root damage is exerted by certain fertilizers. Magistad & Oliveira (1934) observed that "the number of galls on fertilized versus non-fertilized inoculated plants seems to indicate that fertilizer, for some reason or other, has slightly retarded the entry of nematodes into the roots *). The difference in galls per plant is 21.27 ± 18.7".

In an experiment comparing the percentage of tobacco plants infested in untreated lots with the percentage infestation in plants grown in soil fertilized with 200 lbs of blood meal per acre, I obtained confirmatory evidence to the trend of the observations of these authors in that the treated plants showed an infestation of 3.45 ± 0.12 % as against 21.94 ± 0.27 % in the untreated ones. These figures are highly significant statistically and far more convincing than the results of Magistad & Oliveira. Table I (opposite) shows details of this experiment.

As regards the type of soil as a factor in nematode populations little or no experimental data are available, but from

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*) A possible explanation of the fertilizer effect may be that the roots of fertilized plants grow much more rapidly than those of non-fertilized plants with the result that promoted lignification of the roots of the former prevents penetration of larvae at a much earlier stage.

general experience it can be stated that heavy clay and loam soils are usually much less infested than lighter sandy soils (Godfrey, 1926). The assumption seems to be justified that the texture of the heavier soils offers more resistance to the movements of the larvae and causes inaccessibility of the oxygen necessary for their well-being. Linford (1939) found that plant tissues also appeared more attractive in sandy soils and suggested that this might be due either to the freer movements of the nematodes in such soils or to adsorption of the attractive substances by the soil colloids in the case of heavier types, reducing the intensity and extent of attraction.

In South Africa the s.c. "turf" soils *), which must be grouped under the heavy soil types, are seemingly an exception to the above rule, since they are sometimes found to be extremely heavily infested. But, still, the impression is obtained that it takes rather long for a population to be built up and becomes established, especially in the s.c. "black turf".

Different methods of estimating the nematode population of the soil have been elaborated. Those, that will yield a relative nematode index for practical field experiments, will be discussed later (see page 43 et seq.). For the moment we are only interested in the results of calculations that give an impression of the absolute numbers of nematodes present in the soil. Jobert (1878) in Brazil, basing his estimations on the number of eggs per "kyste" (egg mass) and the number of oothecae in coffee trees, arrived at a figure "trop faible certainement et pourtant effrayant, de plus de 30 millions d'Anguillules par caféier ". In a similar way Godfrey (1931) comes to "potentialities for between 2 and 4 000 000 new infestations" from the roots of a cowpea plant. Brown (1933) concludes from microscopic examinations of peat soil that populations of between 8 and 45 000 000 per acre are likely.

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These figures are rather low as compared with the above observations of Godfrey and subsequent investigations by the same author (1936) when he found that in pineapples at least 100 larvae were necessary within an inch of a root tip to produce a terminal gall in that root. In this way he comes to about 7 527 168 000 nematode larvae per acre/ foot (or 172 800 per cubic foot) and he even finds a potentiality of 80 000 larvae from every inch of root in cowpeas, which amounts to a release of 1 600 000 larvae into less than a cubic foot of soil for the entire root system of the plant. For Heterodera schachtii Thorne (1939) finds "the total of 14 795 000 000 (larvae) per acre not an unusually high number for a severely infested field".

The great similarity in the biology of Heterodera schachtii and Heterodera marioni justifies the opinion that such figures are applicable to populations of the latter nematode as well.

Though most investigators (admittedly for practical reasons) restrict their estimates of the soil population to the top foot or so of the field, it stands to reason that - in order to obtain a proper indication of the total number of eelworms in the soil - the depth distribution should be taken into consideration as well. Many attempts have been made to determine this factor, but it seems impossible to lay down general rules. Godfrey (1924) - as far as I am aware - was the first to tackle the question from an experimental basis. He took soil from different depths and tested the nematode content in pots with indicator plants and summarised as follows:

"A considerable variation from one spot to another and from one month to another occurs in the depths of the regions of maximum infestation. Many factors no doubt enter into this, such as (1) differences in depth penetration of infested roots *)

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*) Which naturally varies considerably with the different host plants (H.).

(2) the relationship of time of taking soil samples to a period of heavy rainfall (3) the height of the water table (4) the type of the soil and the subsoil". To these factors could be added the availability of oxygen, subterranean water currents (cf. Thorne, 1942) and capillary flow. Godfrey found nematodes to a depth of 34 inches. Krishna Ayyar (1933), in South India, comes to results in the same order of magnitude (30 inches) and finds a maximum of infestation between 1 and 1½ inches from the surface (cf. also Scott et al., 1939).

Thorne et al. (1942) likewise observed great variations in the numbers of larvae at different depths in fields planted to cotton and alfalfa (lucerne: Medicago sativa) and conclude that the distribution of the larvae appears to be determinable by the location of roots of these plants. Highest populations were found in varying vertical sections, sometimes reaching down to 5 feet, which - these authors remark - "suggested the occurrence of larvae at still greater depths" *). However, such tremendous depths can by no means be taken as general examples.

It will be clear that the absolute nematode population of the soil constitutes a largely varying, rather indeterminable magnitude, which can only be roughly estimated. Not to be confused with the nematode population of the soil is what Christie (1946) correctly calls the "inoculum potential" of the soil, which, he remarks, "may be influenced, no doubt, by many factors, but it is directly dependent, initially, on the egg production of the parasites".

The question of soil population and distribution of the larvae, of course, is intimately connected with the passive movements of the parasites. Especially with respect to the control of eelworm it is of great importance to know how the

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*) Scott (1943) reports on galled peach tree roots that were found as deep as 6 feet.

parasites and eggs are disseminated in order to be able to prevent contagion, to isolate foci of infestation or, finally, to guard against reinfestation of laboriously disinfected soils.

There is a large variety of possible means of spreading inocula of Heterodera marioni. First of all current water should be mentioned. Where irrigation is practiced or flood water is likely to run from infested localities to other areas, care should be taken to prevent dissemination through this medium, bearing in mind the fact that water has been proved to be a natural habitat for both eggs and larvae (Godfrey & Hoshino, 1933; Brown, 1933; Godfrey et al., 1933; Linford, 1941 c). Contour walls and ditches will here serve as useful precautions. In regions where seedbeds are watered from small rivers and streams (as is general practice in the tobacco areas of Southern Rhodesia) the water supply may easily be contaminated by means of surface water from adjacent infested fields *).

If parasitized seedlings are planted out (as, say, in the case of tobacco) to uninfested fields, the farmer himself will be the distributor.

Another common source of eelworm infestation is compost, into which infested roots have been worked up with plant waste.

Owing to the minute size of the eggs and larvae of Heterodera marioni there is always a potential danger of dissemination through lumps of soil from infested areas as may adhere to farm implements, hoofs of horses and oxen or to the shoes of workmen. Seedlings, cuttings, tubers, etc. from nurseries or other farms are likewise to be suspected.

In America the pocket-gopher, a burrowing rodent, was found to be a carrier of nematode inoculum (Young, 1940 b). This

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*) In the tobacco area of Trelawney, Southern Rhodesia, I have observed a gradual down-stream spread of eelworm infestation on farms bordering a small river, the water of which was used for the watering of tobacco seedbeds. Some of these farms, within a year's time, became heavily infested in all the tobacco lands, where eelworm had never been noticed previously.

animal is able to store plant material in its cheek pouches and may in this way transmit parasitized root fragments all over its warren, possibly running into uninfested land.

Kofoid & White (1919), Sandground (1923), Keller (1935) and Bacigalupo (1936) reported on the occurrence of Heterodera eggs in human faeces. Apparently infested vegetable food (like carrots, potatoes) was the source. Kofoid & White, who took these eggs for some unknown human parasite's ova, tried in vain to hatch them. The negative result of their experiments finds an explanation in the following argument.

Although the egg shell, being of a chitinous nature (Chitwood, 1938), cannot be digested and mammalian body temperatures (37 - 38.5° C) are below the minimum lethal temperatures of eggs and larvae *), it is unlikely that viable eggs would pass through the alimentary tract of any mammal as digestive juices and other harmful substances may well diffuse through the egg shell; moreover, the length of the exposure time (in cattle the chymus may take as long as 3½ days to pass the alimentary tract: Ewing & Smith, 1917), lack of oxygen and the very small "safety margin" allowed by the body temperature of such an intermediate host should all be considered as of possible detrimental influence during the time the eggs pass through the intestines. Supposed danger of infection by means of manure, therefore, must be excluded, especially when the manure is well fermented.

Direct information on the longevity of free-living larvae under natural conditions is not available. Brown (1933) found that "some nematodes survived for 12 months in flooded soil" and Franklin (1937) found soil heavily infested with Heterodera marioni still infective after 16 months out-of-doors without any vegetation in it. There is, however, no proof in these observations that it was not the eggs (which - as we have

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seen - remain viable over very long periods) but the larvae that caused the "carry over" of infestation.

Regarding the above question another problem remains, viz. whether or not the eelworms are able to feed during their non-parasitic stage. It is a striking fact that the newly hatched larvae are richly supplied with a granular or globular form of nutriment in the mesenchym which is gradually used up during their activities. But this does not exclude the possibility of food intake, either by means of endosmosis through the integument or via the mouth and alimentary tract. It seems unlikely - to my mind - that continuously active larvae would thrive on this food reserve only for periods of several months. In the laboratory Heterodera larvae can easily be kept alive in water cultures for two or three months on condition that the containers are put in the dark. Without the latter precaution the cultures die out in a couple of days. But whenever an eelworm culture was exterminated, a rich vegetation of Fungi, Bacteria, Protozoa, Algae and sometimes Tardigrada would always be observed and I thus obtained the impression that this over-crowding of their natural habitat and, perhaps, parasitism (see under "Biological Control", page 35 et seq.) more than starvation or any other factor was responsible for the death of the larvae. Plant detritus and humus may serve the larvae as nutriment so that the mesenchymatic granules would be more of an auxillary function. In this connection it is of interest what Nagakura (1930) remarks on observations made by Strubell in 1883 on Heterodera schachtii, writing: "Strubell ist es gelungen, Larven, die er in ein Gefäsz mit humusreicher Erde ohne Pflanzen brachte, gleichfalls in ihre späteren Stadien überzuführen; er fand in solchen Zuchtapparaten sowohl Weibchen von völlig kugeliger Gestalt, wie Männchen, die, fast derartig ausgebildet, in der flaschenartigen zweiten Larvenhülle eingeschlossen lagen, und schliesst daraus: 'das die Einwanderung nicht immer eine notwendige Bedingung für die Entwicklung der

Larven ist'. Es ist möglich, dasz die Larven Strubells den Nahrungsstoff aus der im Humus übrig gebliebenen Pflanzenwurzel saugten und so sich weiter entwickelten. Jedenfalls kann Heterodera radicicola ohne Nahrungsstoff von der Pflanzenwurzel ihre Entwicklung nicht vollenden".

Tyler (1933 c) also remarks that "the larvae do not feed except in living plant tissue" and hence are unable to "grow and develop while free in the soil", though "they can survive for months without food if they are not too active". Thus far no experimental data are available to show whether or not the free-living stage is merely a transitional phase as it is generally believed to be. The plant-parasitic stage, however important and necessary for the propagation of the species (as it includes the sexual phase), might be shown to be really of much less importance than the terricolous part of the life cycle. It is, therefore, not unlikely that entirely new light could be thrown on many present-day discrepancies on the infectiveness of soil and water and the longevity of the larvae.

The origin of Heterodera marioni is concealed in complete darkness since the parasite is a typical cosmopolitan. In Southern Rhodesia it was found in s.c. "virgin" soil, though the writer has often been able to detect certain foci of infestation in places with indubitable traces of former native cultivation, which suggested the human element to be a link in its geographical distribution. However, this gives no clue as to the native country of this agricultural pest.

CONTROL The economic importance of Heterodera marioni due to its threatening expansion in agriculture and horticulture readily explains the fact that eelworm research has mainly been focussed on its control. Two different lines can be followed in this respect: the one not necessarily scientific, but under the stress of daily praxis, in search of quick, practical measures for at least reducing financial loss, the other scientifically aiming at the ideal method

that ensures total eradication of the pest. Either way has its own merits, the difference being mainly a matter of approach. It is, therefore, only in consequence of the scope of this paper as a study that I have stressed the scientific way of attack, without prejudice against other attempts.

One may distinguish between "defensive" and "offensive" control. Under defensive control I list:

a) preventive measures against spread of the disease which logically result from our knowledge of the parasite's means of distribution and propagation;

b) sterilization of seedlings, cuttings, tubers, root-stocks, etc.;

c) the study and practical application of resistant crop varieties.

Offensive control, on the other hand, implies all direct means of soil sterilization, such as:

d) s.c. "agricultural practices" like: desiccation of the soil (bare fallow) with ploughing, harrowing and eradication of weeds, especially of those that are known to be host plants; crop rotation, using immune or highly resistant plants;

e) flooding;

f) soil sterilization by means of heat: steam sterilization; direct application of heat from overground fires; electrical heating (hot-house practice);

g) freezing;

h) catch or trap cropping;

i) biological control;

j) chemical control.

A critical survey of these different means of control will give an impression of their possibilities and limitations.

PREVENTIVE MEASURES AGAINST SPREAD OF THE DISEASE These are more common sense practices which hardly need an experimental footing as e.g. the construction of contour ridges, walls and storm water

ditches to prevent the contamination of uninfested fields by surface water from eelworm lands; the lay-out of irrigation schemes, such that irrigation water will never run from one land to another, unless the top field is absolutely clean; the use only of uncontaminated irrigation water; removal of lumps of infested soil on shoes of workmen, hoofs of animals, farm implements, etc.; prevention of infested plant material being worked up with compost.

STERILIZATION OF SEEDLINGS, CUTTINGS, TUBERS, ROOTSTOCKS, ETC.

It often happens that parasitized plants have to be transplanted or that nursery stock shows signs of infestation. In such cases it is necessary to destroy the nematodes and eggs that are present in the material without killing the host. Hot water or steam is usually applied, but general rules cannot be given for the combination of temperature and time of exposure, which has to bring about the extermination of the parasites without (or at least with minimal) injury to the plants, since the optimal effect of the product temperature x exposure time varies considerably with the plant species to be treated. Successful treatments for different kinds of plants were reported by Byars & Gilbert (1920), Cobb (1929), Brown (1934), Sherman (1935), Goffart (1936), Wade (Proc. of the Root-knot Nemat. Conf., 1938), Chester & Cress (1939) and Burk & Tennyson (1941). The applicability of this method is naturally very much restricted, being mainly a last resort for gardeners and nursery men when valuable plants are concerned, which justify the time and costs spent in this way. Moreover, absolute certainty that all nematode life is destroyed can never be obtained with this treatment.

THE STUDY AND PRACTICAL APPLICATION OF RESISTANT CROP VARIETIES.

A tremendous amount of experimental work has been done by various authors on the problem of breeding varieties resistant or immune to eelworm attack. It is impossible to discuss here

all the claims of success that appear in the literature, so that I will restrict myself to the outstanding features.

The problem is more complicated than would appear at first sight and it seems that many investigators are not aware of all its intricacies. An instance of a simple fact, so often neglected, but still of fundamental importance in selection work for nematode resistance, is discussed by Barrons (1940) as follows: "Insuring a uniform and severe epidemic is a major problem in testing plants for resistance to any disease, particularly in breeding work when making individual plant selections". The principle will be clear if it is realized that the plants from which unbiassed selections are to be made have to be under exactly the same circumstances, which means, in this way, that equal chances of infestation for all plants will only be offered by a uniform, homogeneous distribution of the eelworms. Since this is never realized with natural [illegible]al infestations (see page 16), one has to resort to artificial infestation, which - even under most favourable conditions - will only be an approximation to the ideal. This should be well understood by the selection breeder.

Another, just as fundamental question is this: do the available eelworms themselves represent a homogeneous population? If not so, some of them may be expected to be biassed. This leads us directly to the much disputed problem of <u>biological strains</u>. Considerable evidence has been accumulated leading to the assumption that biological strains occur in the species Heterodera marioni: Tischler (1902), Watson (1924), Bosher & Newton (1933), Sherbakoff (1939), Barrons (1939), Thorne et al. (1942) and especially Christie & Albin (1944) and Christie (1946), whereas negative information on this point (Beasey, 1911; Mallooh, 1923; Fajardo & Palo, 1933) is far less convincing.

Discrepancies in this matter may be propitiated by the views of Steiner (1925) who concluded from critical analysis that the

biological strains of Heterodera marioni are no rigidly limited groups, but rather adaptible categories. "The situation may be best characterized as follows", Steiner writes, "Although the different species of plant parasitic nematodes attack and feed on a wide range of host plants, a given population of one of these species will, if possible, always first attack the kind of host plant that its parents and ancestors lived on. If this plant is not available, in not being present or being already highly parasitized, host plants of any kind of near relationship, taxonomical, chemical, etc., will be sought and attacked. If the ancestors of a population of one of the mentioned nematode species lived during many generations on a number of different host plants, all these host plants and their related forms will be quite easily attacked. The population will then usually be found to be of somewhat polyphagous instinct. But, if the ancestors of a population have lived for a number of generations on a single species or even variety of host plant, their descendants may not attack any other plant known to be a host; or, if they do so, it will be only with difficulty and then only in a small number. It might require generations before a new host is again attacked in the same degree as the old one. But a good part of the population, apparently unable to accommodate itself to the new conditions, starves to death and in an extreme case only a few specimens, or even none, seem to have the ability to accustom themselves to the new host plant. Such nematode populations are monophagous, and because of their specialization on one certain host plant were named "strains" or "biological or physiological races" in analogy to the well known biological or physiological races or strains of fungi, insects, etc. It would, however, be necessary to assume that even in the highest specialized population specimens of still impure genetical constitution are present, from which strains of any kind could be segregated, otherwise cases like "compelled" change of host plant through lack of the preferred species could not be understood".

In line with Steiner's views are those later expressed by Quanjer (1928) who, regarding the possibility of "bridging hosts" for Heterodera marioni (in analogy with observed phenomena in Heterodera schachtii and Tylenchus dipsaci), remarked that "...it must be possible to attract the offspring of a specialized nematode to all the known host plants by changing the host for the succeeding generations in such a way that each following host will be of the closest relationship to the foregoing one. It will even be possible by using the known hosts as "bridges" to infect plants not yet known as hosts".

Considering the problem of resistance in this light, Steiner continues: "Heretofore, if a plant was not found to be infested, it was termed resistant, regardless of the causes, which often were not at all within the plant" (underlining by me, H.)

Tufts & Day (1934) who did extensive work on nematode resistance in various fruit trees, also warn against anticipated conclusions: "In experimental work on this problem one must consider the possibility that in the absence of susceptible host plants, nematodes may attack partly resistant ones; further that in the presence of very susceptible varieties, these parasites may be attracted from the partly resistant hosts". Weinberger et al. (1943) observed e.g. that peach seedlings which showed no galls in the first year became diseased after 2 or 3 years in infested soil.

An illustration of the fact that so called "resistance" is not always persistent towards other nematode populations or under other circumstances of cultivation is given by Watson & Goff (1937) in that "corn, although usually highly resistant on well drained land, is often rather heavily infested on poorly drained land". Personally I have found maize infested with Heterodera marioni in Southern Rhodesia, even on well drained lands, whereas in the Union of South Africa this plant is usually immune and widely used as a rotation crop in agricultural schemes for the extermination of eelworms. (cf. also Anonymus, 1947).

Steiner distinguished sharply (and in my opinion rightly)

between host resistance and host immunity. Host resistant, according to Steiner's definition, are plants which actually resist the entry of the larvae, whereas host immunity *) will be the ability of certain plants to show few signs of suffering in spite of the presence of root-knot galls.

The question arises, however, whether true resistance (in the sense used by Steiner) does really exist. Barrons (1939) concluded from direct observations that "no concrete evidence that the larvae do not enter the roots of resistant varieties has been presented" (cf. Clayton, 1940, and Clayton et al., 1944). "No significant differences were noted", he continues in discussing his experiments, "between the rate of entry in the most resistant and insusceptible plants in the seedling stage".

Thus, according to Barrons, whose observations are confirmed by Smith & Taylor (1947), s.c. "resistance" would be "due to factors entirely within the plant itself that do not exclude the entrance of larvae but either kill the nematode after it enters or cause it to starve **) although it is not impossible that certain morphological root characteristics accomplish or contribute to resistance, it seemed more reasonable to postulate resistance due to certain physiological factors".

Christie (1946) observed that "larvae do not enter the roots of some plants as readily or in as large numbers as they do others and the existence of plants immune (= resistant according to Steiner's definition, H.) to invasion, at least by some races, seems a likely possibility".

The above survey will, no doubt, illustrate the complexity of resistance problem. Typical for all parasitological problems is the refined "lock and key" mechanism, which is here presented.

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*) "Tolerance" may be a more practical word than "immunity" (Barrons, 1939) which (though by no means ambiguous) appears misleading as it is usually wrongly interpreted.

**) Linford (1939) noted that "larvae of Heterodera marioni may fail to distinguish roots of suitable hosts from those that are either difficult to penetrate or inhospitable after penetration" (cf. Steiner's "host preference". Whether the behaviour of the nematodes would be the same if they had the choice, is not mentioned by Linford).

We therefore will be justified to conclude that, unless more unambiguous evidence is produced, many question marks have to be placed behind alleged claims of "resistance" or "immunity".

Additional draw-backs of eelworm control through plant-breeding for resistance are: the taking up of considerable time (experiments of this kind may run over several years); the occupation of much experimental space on the research station, and, last but not least, possibly also the loss of desirable qualities in the crops under experimentation, if genetical factors for true resistance can be proved to exist at all.

AGRICULTURAL PRACTICES Among the s.c. agricultural practices should be mentioned some which - under c[illegible] conditions - will reduce the nematode population of the s[illegible]

Ve[illegible]ive work has been done by le Roux & Stofberg (1935) [illegible]stained best results with bare fallowing (monthly ploughing and hoeing with subsequent weeding) in experiments running over eleven months. Clayton (1940) and Clayton et al. (1944), applying the method of crop rotation and especially studying the question of soil "carry over", showed that only few crops are able to starve out the nematodes. Clayton mentions i.a. Zea Mays as an alleged resistant crop which is sufficiently parasitized to carry over maximum nematode populations". As mentioned before (page 31), my own experience confirmed these observations.

After several years of practice in the control of eelworm I am firmly convinced that crop rotation, being entirely dependent on the resistance of certain crops (and we have seen how questionable such "resistance" may be!), is a very unreliable means of control (cf. Clayton et al., l.c.). It can only be recommended where much land is available and useful crops can be planted in succession. Eradication of weeds, many of which are very susceptible natural host plants, will, of course,

always be a necessary precaution, but bare fallowing *) is out of the question on farms where intensive cultivation has to be practised. Moreover - unless one goes to extremes, so that the profitableness becomes questionable - these methods never result in total eradication of the eelworms (cf. Godfrey & Hagan, 1934) and the soil population is too quickly built up again (cf. King, 1938; Thorne, 1939).

FLOODING Flooding has often been suggested as a means of control, but was never found to be recommendable by those who investigated its possibilities experimentally. From a survey of the literature on this subject Brown (1933) concluded that previous experiments were not continued long enough to determine the time necessary to secure complete control, which, he stated, "would entail the loss of 2 years' crop". His own experiments showed that 4 and 6 months of flooding killed the larvae but not the eggs in the soil. Even 12 months of flooding were survived by some nematodes and only 22½ months exterminated all forms of nematode life.

Watson (1924), however, claimed that 4 - 5 months submergence resulted in complete control, but even this he considered "too long to make the method of much practical benefit".

SOIL STERILIZATION BY MEANS OF HEAT A method applicable only to glasshouses and seedbeds is sterilization of the soil by means of heat. Usually steam is recommended, but also hot water, and, rarely, direct heat from fires on or under the soil. Good results have been reported by Russell & Petherbridge (1913), Melchers (1919), Byars & Gilbert (1920), Godfrey (1923), Newhall (1930), Watson & Coff (1937) and Clayton et al. (1941). There are, however, many objections against this method, chiefly because of the difficulty to obtain sufficient penetration of the heat; the

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*) A method based on the very meritorious work of Godfrey's Hawaiian school (see Godfrey & Morita, 1929; Godfrey et al. 1933; Godfrey & Hoshino, 1933, and Hoshino & Godfrey, 1933).

working expenses are high and adverse effects on soil fertility have been noticed (Godfrey & Hoshino, 1933). Newhall (1935) reported success with an electrical means of heating the soil for sterilization purposes which could find only very limited application.

FREEZING Freezing never seems to have been seriously considered and remarks on its applicability have only been made in passing. Technically the method is more expensive even than heat treatment and the results more questionable.

CATCH OR TRAP CROPPING The problem of eelworm control has been approached from an entirely different angle by making use of s.c. catch or trap crops ("Fangpflanzen", "plantes pièges"): quick-growing, highly susceptible crops, specially planted to attract the eelworms present in the soil, to be subsequently destroyed before a new generation makes its appearance.

This method, initiated and largely advocated by Julius Kühn in Halle, Germany, for combating the sugar-beet eelworm, Heterodera schachtii, met with much less enthusiasm amongst authors experimenting on the control of Heterodera marioni.

The trap crops are usually applied in crop rotation schemes. An extensive literature survey on the subject of trap cropping is given by Godfrey & Hagan (1934) and Godfrey & Hoshino (1934) and a thorough study of its possibilities was made by these authors. Though seemingly promising results were obtained, it appeared that even 16 plantings could not totally eradicate the nematodes. A constant danger remains in those roots that are left behind as reservoirs of new infestations after uprooting of the trap crop. If not properly manipulated, these plants may even increase an originally much lower soil population of eelworms.

BIOLOGICAL CONTROL First introduced by Steiner & Heinly (1922) who discovered certain predatory nematodes amongst Heterodera populations, the method of biological control gained considerable interest in laboratory and field experiments.

The predators pierce the cuticle of their victim and devour it by sucking out its body contents. Linford (1937 b) and Linford & Oliveira (1937) described several such predacious nematodes and studied their feeding habits.

In 1933 Sherbakoff described a fungus predatory on terricolous nematodes (a similar fungus is said to have been pictured by Wilhelm Zopf as early as 1888). By means of "lasso's" the mycelia of the fungus snare the eelworms which are then devoured by ingrowing haustoria. These discoveries were followed by similar observations by Drechsler (1937) and others. Especially the Hawaiian school of Linford contributed much to our present-day knowledge on biological control. In 1938 Linford & Oliveira described 52 enemies of Heterodera marioni, viz. 11 nematode-trapping fungi, one egg parasite, 6 non-trapping parasites, one ecto-parasitic protozoan, 24 predacious nematodes, 6 mites and 3 predacious tardigrades. Laboratory experiments with the aim of initiating practical applications were carried ou. by Linford & Yap (1939), Deschiens (1941) and Deschiens et al. (1943); subsequent greenhouse and field tests by Linford (1937 c), Linford & Yap (1938) and Linford et al. (1938) showed that increased activities of both nematode-trapping fungi and predacious nematodes were induced by dressings of pineapple choppings and compost (see also Stewart et al., 1928, and Watson, 1943). Though eelworm infestations could be significantly reduced, these authors are well aware of the limitations of biological control. Throughout such experiments one soon meets with the general law of biotic equilibrium which results from interference of the biotic potentials of both prey and predator or host and parasite. This dynamic equilibrium puts a dead stop to the extent of control obtained in this way. Nevertheless, as an auxillary measure in the whole program of eelworm eradication, biological control may play an important role. It is very likely that the frequently observed advantageous use of compost in agricultural and horticultural practice is largely due to its initial support to the biotic resistance of eelworm populations.

CHEMICAL CONTROL Of all possible means of eelworm control chemical applications undoubtedly top the list for effectiveness, profitability and practicability. The ideal compound should be highly toxic to eelworm, innoxious to plants, animals or human beings, without adverse influence on the qualities of the soil, easy to apply, low in price and, above all, while satisfying these conditions, it should bring about total eradication of the parasites.

It is not within the scope of this paper to endeavour in a complete survey of all chemicals and mixtures, which, up to the present time, have been tried out for possible vermicidal properties by various research workers all over the world. Their number is legion and, I dare say, practically every disinfectant or any substance with the least detrimental faculties one could think of has been put to the test, either in laboratory experiments or in pot, greenhouse or field trials.

There is no difficulty in finding a compound that rapidly kills eelworms. But eelworm killing is somewhat different from control of eelworm in the soil. Reducing the population of plant-parasitic nematodes is one thing, but to obtain total eradication is a different story.

After a closer study of the more promising means of chemical control discussed below, it appears that only a few are able to pass the examination satisfactorily, as some factor or other usually cancels out the general advantages.

Nevertheless, at least some results have been obtained, which, under special circumstances and on certain conditions suit the demands of the praxis. But no present-day chemical allows for generalization of its applicability, so that we still have to admit that the ideal compound has not yet been found.

The greatest obstacle on the way to absolute control seems to be that, unless all infested roots are totally decayed, no known chemical reaches all the nematodes and eggs in quantities sufficient to kill them. Reinfestation from such reservoirs,

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therefore, is always likely to occur, sometimes even very rapidly (cf. Thorne, 1939). In view of this fact one could postulate another condition for the ideal means of control, viz. it should be such, that it can be easily and economically applied several times in succession. This very important condition - I am afraid - cannot be put up with any of the classical means of chemical control where the vermicidal effect is largely dependent on their being administered in bulk.

With a few examples I may illustrate this point. I selected from the literature on this subject only the most prominent achievements in eelworm control.

It should be realized that meeting the requirements of a practicable nematocide is easiest accomplished with pot experiments, more difficult with hothouse work and seedbed trials, whereas general field practice yields most of the troubles for the practitioner. The ideal nematocide would be equally well suited for all circumstances of application.

The cyanides NaCN, KCN and $Ca(CN)_2$ have often been under investigation. They are all readily soluble salts and can be broadcast and ploughed in or otherwise be mixed with the soil as the circumstances may indicate.

Duruz (1917) recommends 200 lbs per acre of NaCN for greenhouse soil treatment, Robson (1919) applied 1000 lbs per acre of NaCN or KCN and Miles & Turner (1928) claimed that $Ca(CN)_2$ at the rate of 750 - 2000 lbs per acre gave 90 % control, against which Young (1939 a) reports negative results with 1200 lbs per acre of NaCN. These results are very contradictory and the method seems to be hardly recommendable as yet.

More success could be expected from liquid HCN injected into the soil, but no positive results were ever obtained. The gas is very volatile, inflammable and extremely poisonous and for that reason alone not recommendable in its pure form. A very much safer way of liberating HCN in the soil is by means of a chemical reaction between sodium or potassium cyanide and

ammonium sulphate, $(NH_4)_2SO_4$. The two salts are successively introduced into the soil by means of irrigation water so that a gradual flow of the gas is secured as the chemicals mix. Krishna Ayyar (1933) reports good results in pots with 800 lbs of KCN and 1000 lbs of $(NH_4)_2SO_4$ per acre, but Byars (1919) obtained disappointing results with quantities as large as 3600 lbs of NaCN and 5400 lbs of $(NH_4)_2SO_4$ per acre (being 9 times the popularly recommended rates!). Again Watson (1924) considered the method practical (applying only 1½ times the "standard" dosage), though very expensive. These results obviously will not strengthen the faith in cyanides as vermicides in soil.

Calcium cyanimide, although it is no cyanide, may be mentioned in relation to the cyanides. It has good qualities as a fertilizer but as a nematocide it appeared useless even in dosages up to 4000 lbs per acre (Taylor, in Proc. of the Root-knot Nemat. Conf., 1937 and 1938; Young, 1939 a, 1940 a, b; Godfrey & Young, 1943). Still, Krishna Ayyar (1933) claims to have obtained complete control with 1000 lbs per acre in pots.

Two more salts should be considered, although they seem to have found only little attention, viz. sodium nitrite ($NaNO_2$) and ethyl mercury iodide. The former was used by Feldman & Shaw (1943) in greenhouses at the rate of 3630 lbs per acre when absolute control was obtained, and by Ellis (1943) who applied it in field experiments, giving 98.6% control at the rate of 2420 lbs per acre. More information would be necessary for proper judgement, but the quantities used are rather high! Ethyl mercury iodide (contained in the strength of 5% in Du Bay 1155-HH, a proprietary compound) was successfully used in pots by Lammerts (1940) at the rate of 195 - 292 lbs per acre (2 - 3 grams per square foot), who claimed to have obtained absolute control. These quantities are admittedly low, but elaborate field experiments should be carried out before this substance could be recommended.

With the above mentioned chemicals all the solid compounds that come into consideration have been discussed.

An entirely different practice has been evolved with volatile liquids which liberate toxic fumes when injected into the soil (soil fumigation) *). The classical example of this is carbon bisulphide (carbon disulphide, CS_2) which is applied either pure or as a watery emulsion. The emulsion was first introduced by Schaffnit & Weber (1929). In pot experiments, Chapman & Parker (1929) obtained total control with 43560 gallons per acre of a 0.7 % CS_2 emulsion. Guba (1932) comes to figures of 392 dollars per acre for 95.71 % control in greenhouses and claims to have achieved complete eradication with two such applications. Comparatively good results with CS_2 emulsions were also reported by Taylor (see Proc. of the Root-knot Nemat. Conf., 1937 and 1938). Other investigators used the pure chemical by various means of application, with or without gas-tight covering or water seals in quantities from 750 to 3000 lbs per acre (Newhall, 1930; Godfrey, 1935 a; Taylor, l.c.; Young, 1939 a, 1940 a, b; Godfrey & Young, 1943) ranging in effectiveness from 48 - 100 % control. It will be clear that the emulsion form is much more economical than the pure chemical, but a disadvantage is the enormous quantities of water required. Direct CS_2 injection, apart from being more expensive on account of the larger quantities of the chemical, requires also very much time and involves (for best results) extra costs of injectors and surface covers. For field work, therefore, this chemical is definitely uneconomical. Moreover, it is difficult to handle and dangerous in being very volatile and liable to explode.

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*) Liquid HCN, mentioned above with the cyanides because of the chemical relationship, should actually be classified under the soil fumigants.

In analogy with HCN, CS_2 may also be liberated in the soil by means of chemical reaction. Potassium xanthate ($KS_2COC_2H_5$) e.g. changes in acid medium into xanthic acid which gradually decomposes into CS_2 and alcohol (de Ong, 1[illegible]). Fair results were obtained by de Ong & Tyler (1928), though, according to these authors, "eradication cannot be expected, only varying degrees of control for one or two years" (see also Godfrey, 1935 a).

For many years the most outstanding soil fumigant has been liquid chloropicrin (CCl_3NO_2, sometimes called "tear gas" *). This gas is undoubtedly a very effective nematocide (Johnson & Godfrey, 1932; Godfrey et al., 1934; Godfrey, 1935 a; Neller & Allison, 1935; Newton et al., 1937; Taylor, see Proc. of the Root-knot Nemat. Conf., 1937; Young, 1939 a, 1940 a, b; Taylor & McBeth, 1941 b; Newhall & Stark, 1942; Taylor, 1943) but has many disadvantages as well. It is difficult to handle, being a very volatile liquid that liberates poisonous fumes; it is irritating to the skin and in its gaseous form strongly lachrymatory. Gas masks have to be worn while operating with chloropicrin and gas-tight covers have to be applied directly after introducing the chemical into the soil (Godfrey, 1934 b). Chloropicrin has the advantage of being not inflammable or explosive, but is again very toxic to foliage. It is by no means cheap and has, in common with CS_2, all the troubles of soil injection.

Some economizing in the use of chloropicrin may be achieved by mixing it with ethylene dichloride, CH_2ClCH_2Cl, (Chitwood, 1940, 1941; Taylor & McBeth, l.c.; Newhall & Stark, l.c.). Ethylene dichloride alone, strangely enough, seems to be rather useless according to Taylor & McBeth (l.c.) **). Moreover it is quite expensive (Newhall & Stark, l.c.) and highly toxic

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*) Ordinary tear gas, however, is chloracetophenone, $C_6H_5COCH_2Cl$.

**) Godfrey & Young (1943) estimate its lethal dosage between 1000 - 2000 lbs per acre.

as a gas, which implies the necessity of gas masks to be worn when handling it.

Promising results are reported from methyl bromide, CH_3Br, which is also contained in "Dowfume Br 10", a proprietary compound, in a 10 % concentration. It should be applied under gas-tight cover (Taylor & McBeth, 1940, 1941 a) or water seal (Gingrich & Haenseler, 1941 *)). It is toxic to human beings, so that the wearing of gas masks is indicated. An advantage of methyl bromide seems to be its ability to penetrate undecayed roots (Stark et al., 1944), but only data from greenhouse experiments are available.

In recent years much attention has been drawn in the United States to a mixture of 1, 3-dichloropropylene and 1, 2-dichloropropane, best known under the trade name of D-D Mixture. It is reported as the equal of chloropicrin in effectiveness but with the advantages of being cheaper and easier to handle. This compound was introduced by Carter (1943, 1945). Good results were obtained by Stark et al. (1944) in greenhouses; by Parris (1945) in field experiments with surface wetting; McFarlane & Matsuura (1947) who claimed that no soil covering is necessary; and finally by Smith & Taylor (1947) who obtained 100 % control with 512 lbs per acre.

D-D Mixture is a liquid and has to be injected into the soil. This is a draw-back for application in the field, though farming machinery has been constructed that may overcome this difficulty. In South Africa experiments are carried out with this chemical that seem to give promising results. However, before general recommendations can be made, more data should be obtained and the costs should be considerably reduced.

Soil fumigation appears to have certain advantages for small

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*) These authors, carrying out greenhouse experiments, determined the dosage-time curve for complete control as a rectangular hyperbola ($x.y = c = 3$; when x = dosage in cc per cub. foot and y = exposure time in hours). This, of course, is a very favourable feature as it would mean that fairly small dosages should be effective on condition that the fumes can be sufficiently long retained in the soil.

areas like greenhouses or seedbeds. For large-scale control it is definitely unpracticable and too costly *). It is significant that the application of soil fumigants is most widely practised in the United States, a highly industrialized country, where the production costs of the necessary chemicals (often easily available waste or by-products of some chemical production factory), as well as their transport, are comparatively low, and furthermore intensive cultivation of high-priced crops is another factor making soil fumigation profitable even at fairly high costs, if necessary. In a country like South Africa, where large areas are to be disinfected, where chemicals are difficult to obtain and very expensive, fumigation will never be a generally profitable means of eelworm contro.

THE NEMATODE INDEX With all practical work on eelworm control, be it by way of pot, greenhouse, seedbed or field experiments, one is faced with the problem of finding a reliable means of evaluating the number of eelworms that originally infested the soil, as well as the number of eelworms killed by the agent(s) under investigation.

Carrying out laboratory tests one deals with a certain number of larvae which are directly seen under the binocular microscope and the number killed can be accurately counted. In the soil, to the contrary, the eelworms are not visible, so that counting is out of the question. Moreover, their numbers are far too large and all that is possible, is to estimate the population as accurately as circumstances permit.

Cobb (1918) elaborated a method of estimating the nematode population of soil by means of direct sampling. A number of "representative" soil samples was selected from over the area under investigation and thoroughly mixed afterwards. Out of this composite sample aliquot sub-samples were taken, from which the

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*) Though "spot treatments" may reduce the costs considerably (Taylor & McBeth, 1941 b), this naturally yields no 100 % control, but at its best will check invasion at the time the plants are growing.

nematodes were extracted, identified and counted. In this way only a mean density of population can be estimated, so that information on the actual population is very much restricted. More information can be obtained if the procedure is slightly altered to the effect that random samples can be taken over the infested area and the nematodes counted in each sample. The smaller the samples will be and the larger in number too, the more accurate will be the estimation. However, for obtaining data on the distribution of the densities of population a very large number of samples would be necessary which makes the method absolutely impracticable, except, perhaps, for very small plots. Moreover, heterogeneity of densities causes the estimate to be largely dependent on the unit of volume chosen, and this is a very weak point in the process.

The method finally came into discredit as a result of its being far too laborious for ordinary routine work, also because of the danger it implies of considerable sampling errors and the fact that only a skilled eye is able to identify the different species (cf. also Godfrey, 1934 a).

Knowing the practical impossibility of direct sampling as a means of estimating Heterodera populations in soil, the majority of investigators took refuge to measurements of damage caused by the parasites in their host plants. In this way, however, the relation between the real effect of the toxic agent and what is actually estimated becomes rather vague.

This gap in our knowledge on the infestation problem must have been realized by the eminent Hawaiian school of nematode research workers, who tried to overcome the difficulty by correlating gall counts on the roots of indicator plants, the number of larvae within the roots and the percentage of plants infested (Godfrey, l.c.), as well as the number of terricolous larvae in close proximity to the roots of the host plant and the number of galls per plant (Godfrey, 1936). Nevertheless a remarkable taciturness on this point is characteristic of the

literature elsewhere and the actual problem of the missing link has never been explicitly stated or sufficiently analysed theoretically and statistically. The correctness of measuring plant injury instead of directly determining the actual soil infestation is usually taken for granted.

In an effo t to obtain a relative nematode index I worked on a system of classification or grading of root damage (Hovy, 1939), proceeding as follows. An estimate was made of the percentage of roots infested in each plant and the plants thus graded were grouped in classes of 10 % interval. The number of plants in each class was then multiplied by the class weight (the midpoint of the interval) and the figures obtained in this way were summed and divided by the number of infested plants. One thus arrives at a figure, which - for sake of simplicity - from now on will be represented by the letter A. It denotes the average (individual) infestation of the parasitized plants, expressed in percentage of roots infested. If further the proportion of plants infested is denoted by B, one obtains after multiplication of A by B a figure expressing the average infestation per plant.

Chitwood (1941) applied this method in a similar way, but used 5 classes instead of 10. The "root knot index" which he evaluated was derived by taking "the total score of the plants divided by the number of plants".

Smith & Taylor (1947) used only 4 classes (intervals of 25 % root infestation) and worked out their "disease index" by applying what is called by them the "McKinney formula as modified by Horsfall & Heuberger (1942)" which they represent as

$$\text{disease index} = \frac{\Sigma(\text{category numbers})}{\text{number of plants} \times 4} \times 100$$

The best general appearance for this formula would be

$$I = \frac{\Sigma(f_i w_i)}{N}$$

in which I = the nematode index; f_i = frequency in each class; w_i = the class weight = $\frac{1}{2}(m_i + m_{i-1})100/r$ when m_i is

the class number and r the number of classes; N = the total number of plants.

If now n denotes the number of plants infested, it will be seen that in the above notation the writer's expressions become:

$$A = S(f_1 w_1)/n \text{ and } B = n/N \text{ or } AB = S(f_1 w_1)/N$$

Smith & Taylor do not quote the earlier literature (Hovy, 1939; Chitwood, 1941), so that it seems likely that the idea of evaluating a nematode index in this way was arrived at independently by the different authors. McKinney's paper appeared in 1923 (Journal of Agric. Research (U.S.), 26, 195 - 217) but was unknown to me.

The reasoning on which this method is based is as follows. Suppose we would have two plots X and Y with 100 plants each, having 20 % and 80 % of their plants infested respectively, so that $B_x = 0.20$ and $B_y = 0.80$. Comparing the two values of B, one would take Y to be heavier infested than X. But the 80 % of the plants infested on plot Y may be slightly parasitized and the 20 % on plot X heavily attacked, so much so, that the number of nematodes which invaded the plants of plot X might be much larger than that of plot Y. Direct comparison of the factors B (as is usually done by most investigators), therefore, cannot be justified; neither would, obviously, comparison of the factors A be permissible. By taking the product AB, however, we have a figure indicating what the infestation per plant would be, if all the nematodes available in the soil of a given plot would be equally distributed over all the plants grown on that plot; AB thus represents the mean infestation.

This, at first sight, seems to be a reasonable criterion, though up to the present time no proof has ever been given of its correctness.

To this effect I tried first of all to obtain a clearer insight of the phenomena concerned with root damage in order to solve this problem of fundamental importance in eelworm control.

It then had to be investigated, primarily: whether the above index (AB) gives a reliable estimate of the population mean, and secondly: in how far is an eelworm population in the soil determined by its mean, in other words: is the mean the sole characteristical parameter of the population?

The theory of statistics describes a population in terms of its "distribution". Here the word distribution has a very specific meaning that has no reference to the propagation or means of dissemination of the population members. In fact it denotes the spread of a "spectrum" of probabilities *) in which the constant parameters of the population appear in a characteristic, concise mathematical form, the s.c. distribution law.

Since previously we had to admit the uncertainty principle in detecting the eelworm in soil and recourse had to be taken to some indirect information on their presence (in casu: the observation of damage inflicted upon the roots of certain host plants) it should be worth while investigating whether a probability law could serve as a useful substitute for direct observation. This means that, if some distribution law would prove to fully characterize the eelworm population of the soil and its parameters could be suitably evaluated from the available evidence of the parasites' presence, the problem of deriving a reliable nematode index would be solved.

ANALYSIS OF THE PROBLEM Fitting a distribution law to empirical data is a fascinating but somewhat hazardous job. The danger lies in the fact that one can easily be misled if it is not realized that a "good fit" does not give any proof at all of the correctness of the assumption that that particular law of distribution is the only valid explanation of the "behaviour" of our data. Its formula

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*) Regarding the mathematical concept of "probability" I wish to refer to Kerrich (1941).

Table II

class		1	2	3	4	5	6	7	8	9	n	N-n	N
treatm.	I,1	102	392	257	91	19	3	3			867	318	1185
	2	69	106	45	19	2	0	1			242	1349	1591
	3	65	76	32	18	7	0	0	0	1	199	466	665
	4	57	125	31	17	11	3	1			245	343	588
	5	121	82	37							240	1576	1816
treatm.	II,1	10	16	9	2	2	0	0	0	1	40	599	639
	2	28	54	24	12	2	1	0	2		123	1013	1136
	3	29	48	22	18	3					120	615	735
	4	29	44	12	3						88	367	455
	5	4	15	6	1	0	1				27	52	79
treatm.	III,1	8	6	1							15	1083	1098
	2	43	95	15	4	1					158	279	437
	3	43	49	8	7	1					108	730	838
	4	50	52	7	2	2	1				114	738	852
	5	8	15	9							32	463	495
treatm.	IV,1	61	143	68	54	12	5	2	1	1	347	230	577
	2	116	242	100	37	8	3	1			507	665	1172
	3	97	201	76	30	9	5	2			420	668	1088
	4	46	135	38	34	8	9	1			271	248	519
	5	148	218	45	9	3	2				425	367	1792
treatm.	V,1	73	258	210	97	37	18	4	2	3	702	294	996
	2	20	40	10	7	1	0	1			79	1321	1400
	3	191	317	126	48	10	2	4	1		699	1356	2055
	4	41	147	92	60	13	3	2			358	135	493
	5	34	110	57	30	12	5	3			251	105	356
treatm.	VI,1	22	41	14	10	3	1	1			92	925	1017
	2	95	333	133	40	5	1	1			608	315	923
	3	43	109	35	25	12	3	0	0	1	228	884	1112
	4	32	149	85	33	13	6	5	2	4	329	208	537
	5	48	122	92	30	5					297	78	375

total: 27021

For details on classes and symbols see text.

provides a description, nothing more, one that may or may not satisfy the experimentor. Granted that certain generally accepted tests of significance have become established, a large amount of subjectivity nevertheless adhere to the assumed law: first of all, because another probability distribution may be found to be just as well descriptive of the same data; secondly as a result of the explanation of the parameters, which - if possible at all - is often rather speculative; and finally since a "good fit" is no warranty that the law will hold under all circumstances.

The point, therefore, at which the investigator should be satisfied, is rather arbitrary, and one soon appears to be involved in an endless task.

To illustrate this, let us consider the data of an experiment that supplies a varied amount of information on root damage caused by the larvae of Heterodera marioni.

A piece of ground, known (from previous test crops) to be well infested with eelworms, was divided into 30 plots measuring 3 x 15 feet and separated by strips 1 foot wide. These seed beds were sown with a Virginian type of tobacco (Amarello) and wetted by means of watering cans with water from a guaranteed uncontaminated source. Six different treatments were given to the soil, each one with five replications in a randomized block system. The nature of the treatments, for the present moment, should be immaterial to us.

After about four months' growing, the seed beds were soaked with water and the root system of each seedling carefully washed from the mud in order to prevent loss of galled roots as far as possible. The plants were then taken to the laboratory and individually inspected with the roots floating in clear water contained in a glass dish and graded according to the method described on page 45.

Table II (opposite) shows the data obtained in this way. In this experiment an extra class of root infestation was added

as many plants showed very slight attack which enabled a finer distinction to be made between non-infested and the first 10 % interval. The class numbers thus come to denote: 1 = 0 - 5 percent root infestation; 2 = 5 - 15 %; 3 = 15 - 25 %; etc., so that the midpoints or class weights become: 2.5 %, 10 %, 20 %, etc., up to 80 % (higher infestations did not occur).

The data of table II show us now 30 different frequency distributions of root damage to which a distribution law has to be fitted. The nature of these distributions is continuous, which restricts us in our choice of probability law in that the discontinuous types, such as the binomial and Poisson series, the negative point binomial (Greenwood & Yule, 1920) or the contagious distribution (Neyman, 1939) and the like are straight away excluded. Pearson's family of distributions, however, could possibly offer a suitable probability law, so that I started to compute the criteria (see Elderton, 1938) which surely facilitate a search for the best fitting curve and so obviate much unnecessary work.

Evaluation of these criteria (k) implies the calculation of the first four moments ($m_1 - m_4$) and the first two seminvariants b_1 and b_2.

As an example I give here the calculations for the 5 replications of treatment VI. The symbols denote: x_1 the class midpoints (weights); f_1 the corresponding frequencies as observed; m_1', m_2', m_3', m_4' the first four moments about the origin; m_1' the mean and m_2 the variance; m_3 and m_4 the third and fourth moment about the mean respectively.

In table III the totals of columns 6, 7, 8, 9 divided by n (the number of plants infested) give us the first four moments about the origin of the curve for root damage. The moments about the mean are found by the following formulae:

$m_2 = m_2' - (m_1')^2$; $m_3 = m_3' - 3m_1'm_2' + 2(m_1')^3$; $m_4 = m_4' - 4m_1'm_3' + 6(m_1')^2m_2' - 3(m_1')^4$.

The seminvariants are $b_1 = m_3^2/m_2^3$ and $b_2 = m_4/m_2^2$.

In order to obviate unduly large figures the x_i have been divided by 10. This is permissible as it does not cause any difference in the final results. Moreover, it has the advantage that a very simple check on the arithmetic can easily be performed. If e.g. $0.1x_i = z_i$ and $(z_i - 1) = y_i$, the 10th columr of table III gives the values of $f_i(y_i^4)$. Summation of these values gives $\Sigma f_i(y_i^4)$ and on expanding this we have:

$$\Sigma f_i(y_i^4) = \Sigma f_i(z_i - 1)^4 = 1\Sigma f_i(z_i^4) - 4\Sigma f_i(z_i^3) + 6\Sigma f_i(z_i^2) - 4\Sigma f_i(z_i) + 1\Sigma f_i$$

In other words: if we multiply the sums of the columns 5, 6, 7, 8 and 9 respectively by the factors +1, −4, +6, −4 and +1, the sum of these products should check up against the sum of column 10.

For treatment VI, block 1, we have now, table III:

z_i	z_i^2	z_i^3	z_i^4	f_i	$f_i(z_i)$	$f_i(z_i^2)$	$f_i(z_i^3)$	$f_i(z_i^4)$	check
0.25	0.06	0.02	0.004	22	5.50	1.38	0.34	0.09	6.96
1.00	1.00	1.00	1.00	41	41.00	41.00	41.00	41.00	0.00
2.00	4.00	8.00	16.00	14	28.00	56.00	112.00	224.00	14.00
3.00	9.00	27.00	81.00	10	30.00	90.00	270.00	810.00	160.00
4.00	16.00	64.00	256.00	3	12.00	48.00	192.00	768.00	243.00
5.00	25.00	125.00	625.00	1	5.00	25.00	125.00	625.00	256.00
6.00	36.00	216.00	1296.00	1	6.00	36.00	216.00	1296.00	625.00
				92	127.5	297.38	956.34	3764.09	1304.96

check: 92−510.0+1784.25−3825.38+3764.09=1304.96

For the moments about the origin and those about the mean we then find:

$m'_1 = 1.39$ $\quad (m_2)^3 = 2,26$

$(m'_1)^2 = 1.92$ $\quad m'_3 = 10.40$

$(m'_1)^3 = 2.66$ $\quad m_3 = 2.28$

$(m'_1)^4 = 3.69$ $\quad (m_3)^2 = 5.20$

$m'_2 = 3.23$ $\quad m'_4 = 40.91$

$m_2 = 1.31$ $\quad m_4 = 9.47$

$(m_2)^2 = 1.72$

and for the seminvariants: $b_1 = 2.30$ a $b_2 = 5.50$

The criterion is evaluated as

$$k = \frac{b_1(b_2 + 3)^2}{4(2b_2 - 3b_1 - 6)(4b_2 - 3b_1)} = -1.45$$

Similarly the necessary parameters for treatment VI, block 2, are found to be:

m_1'	= 1.27	m_3'	= 5.16
$(m_1')^2$	= 1.62	m_3	= 6.71
$(m_1')^3$	= 2.06	$(m_3)^2$	= 45.01
(m_1')	= 2.62	m_4'	= 14.64
m_2'	= 2.23	m_4	= 2.41
m_2	= 0.64	b_1	= 1.74
$(m_2)^2$	= 0.41	b_2	= [illegible]
$(m_2)^3$	= 0.26	k	= [illegible]

Treatment VI, block 3, yields:

m_1'	= 1.47	m_3'	= 11.93
$(m_1')^2$	= 2.17	m_3	= 2.67
$(m_1')^3$	= 3.19	$(m_3)^2$	= 7.10
$(m_1')^4$	= 4.70	m_4'	= 51.4
m_2'	= 3.54	m_4	= 13.20
m_2	= 1.37	b_1	= 2.74
$(m_2)^2$	= 1.89	b_2	= 6.99
$(m_2)^3$	= 2.59	k	= -15.02

Treatment VI, block 4, again:

m_1'	= 1.78	m_3'	= 21.63
$(m_1')^2$	= 3.15	m_3	= 5.62
$(m_1')^3$	= 5.59	$(m_3)^2$	= 31.63
$(m_1')^4$	= 9.93	m_4'	= 118.32
m_2'	= 5.11	m_4	= 31.49
m_2	= 1.96	b_1	= 4.23
$(m_2)^2$	= 3.82	b_2	= 8.23
$(m_2)^3$	= 7.48	k	= -2.97

Finally we obtain for treatment VI, block [illegible], the following constants:

m_1'	= 1.44	m_3'	= 6.70
$(m_1')^2$	= 2.08	m_3	= 0.41
$(m_1')^3$	= 2.99	$(m_3)^2$	= 0.17
$(m_1')^4$	= 4.31	m_4'	= 17.86
m_2'	= 2.84	m_4	= 1.69
m_2	= 0.76	b_1	= 0.38
$(m_2)^2$	0.58	b_2	= 2.91
$(m_2)^3$	= 0.44	k	= -0.24

Judging by the values of k, it will be observed that the frequency distributions of VI_1, VI_4 and VI_5 would suggest the fitting of a type I distribution, while for VI_2 a Pearson type VI would be indicated and for VI_3 a type III curve.

At this stage it is hardly necessary to say that further persuit of this line would be totally fruitless as even within the same treatment no universal distribution law can be detected. Moreover, the Pearson distributions require a rather large number of parameters which in too many cases would imperil the process of fitting (see page 73). Besides, the method of moments is not a very accurate means of curve fitting, so that, for our purpose, the Pearson types of frequency distribution have to be totally discarded.

After many other unsuccessful attempts I came to the general conclusion that tackling this problem by means of haphazard curve fitting is absolutely useless. It is, actually, a starting from the end. But owing to lack of a priori knowledge regarding the behaviour of the larvae in soil there seemed to be no way out.

Only one opening remained, viz. a trial through inductive reasoning.

Inspired by Prof. J. Neyman's (1939) brilliant mathematical attack on some entomological and bacteriological problems which resulted in his formulation of a new class of "contagious"

distribution, I embarked on an experiment in mind ("Gedanken-eneksperiment").

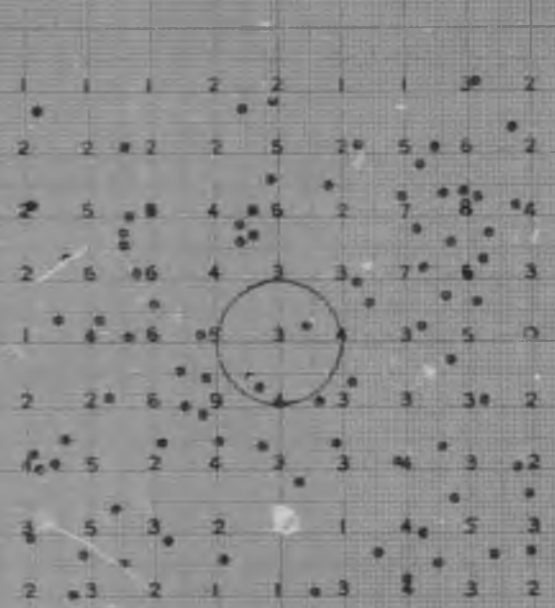
Let us consider a certain volume of soil (V) in which a number (e) of oothecae is distributed at random. In a given time (t) an average number (l) of larvae will have hatched from each egg mass, so that at that time V will contain el larvae. We assume that, as the larvae hatch, they start crawling about in the soil at random. In the time t (reckoned from the beginning of hatching) the eelworms will have been able to travel a maximum distance (a) away from the original egg mass. Since not all the eggs hatched at the same time, there resulted a gradual release of l eelworms from each package during the time t. These l larvae, therefore, will occupy the volume of soil contained in a sphere of radius a around each one of the egg masses. As the spheres of nearby egg masses may intersect there will be an uneven distribution of eelworms in the volume of soil under consideration (V).

Two questions arise now: 1° what kind of distribution of the larvae will result; and 2° how can this distribution be expressed in suitable terms?

To start with the second question, it would be best first to arrive at a practical expression of eelworm density (or concentration) and then to see how the densities are distributed. To divide V into little cubes of standard units of volume will be meaningless, since in that case the densities will be dependent on that (arbitrary) unit and vary with its size. Moreover, one would like to come to an idea of eelworm concentration in every point, throughout the volume V.

As the eelworms move about and a is very small, we may take the concentration in each sphere to be homogeneous throughout the volume $4/3\pi a^3$ and of the order $\frac{3}{4}\frac{1}{\pi a^3}$. This density can now most conveniently be taken as unit, so that, when in a certain point, we find an intersection of 2 spheres, we have a density of 2; if 3 or more spheres are intersecting, the density will be accordingly. In other words, we will be able to express

FIG. I



THE CIRCLE IN THE CENTRE
HAS A RADIUS a.
FOR SAKE OF SIMPLICITY, NO
MORE CIRCLES HAVE BEEN DRAWN.

the eelworm density in every point of the volume V in whole numbers or multiples of the unit value $\frac{3}{4\pi a^3}$ or - which is still simpler - in terms of the number of oothecae found in a sphere with radius a around the point in question.

Figure 1 shows a two-dimensional simplification of the above argument and may be considered as an illustration of a cross section through V. The red dots, jotted down at random, represent egg masses, the circles with radius a are drawn around the points of which the density is required and by counting the number of red dots in each of these circles, we find the density as indicated in their centre points. It should be realised that these figures denote densities in these spots only and not of the whole area of the corresponding circles, since the density naturally varies from point to point throughout the square A.BCD.

If now (as shown in table IV) a frequency distribution is drawn up of the points considered in fig.1, we observe a very good fit with the Poisson series (P = 0.68, which is highly significant).

Table IV.

x_i	obs. f_i	exp. f_i	diff.	$\frac{\text{diff.}^2}{\text{exp.}f_i}$	contrib. to χ^2
0	1	2.96	1.96	$\frac{3.84}{2.96}$	1.29
1	10	9.80	0.20	$\frac{0.04}{9.80}$	0.00
2	21	16.22	4.78	$\frac{22.85}{16.22}$	1.41
3	18	17.88	0.12	$\frac{0.01}{17.88}$	0.00
4	11	14.80	3.80	$\frac{14.44}{14.80}$	0.98
5	9	9.80	0.80	$\frac{0.64}{9.80}$	0.07
6	7	.			
7	2	9.54	1.46	$\frac{2.13}{9.54}$	0.23
8	1	.			
9	1	.			

$\chi^2_6 = 3.98$ P = 0.68

x_i = density f_i = frequency

Theoretically a Poisson distribution might have been expected a priori, but here is at least some evidence of this

being the case.

Though a is dependent on the unknown t and l is indeterminate, it should be borne in mind that the densities expressed in units $\frac{3}{4\pi}\frac{1}{a^3}$ are only relative figures, independent of the true value of the unit, so that the advantage of this concept still remains in the fact that we now deal with natural units and not with arbitrary, artificial ones as would be the case when actual numbers of eelworms had to be related to other units of volume. Furthermore, when remembering that accurate counts of eelworm larvae in the soil were impossible, this appears to be a quite practical unit as will be seen presently.

Returning to our unit of soil (V), one may ask what would happen when a number of growing root tips travel through it. At different points in V these rootlets will meet with varying eelworm concentrations so that (after penetration of the larvae into the roots) galls will be formed that contain correspondingly varying numbers of eelworms. Allowing for a certain amount of chemotaxis, but realising that invasion does not take place in every point of V (so that a number of larvae - while limited in their range of travelling - may not find the host) we come to the conclusion that a root system, developing in V, will take a random sample (in the form of root knots) of the eelworm population in V, according to the distribution of its densities. In other words: the distribution of eelworms in the galls of that root system will represent a true picture of the eelworm population of V which we should be able to estimate from it.

If, therefore, the distribution of eelworm densities is a Poisson series, the distribution of eelworms in the galls should also be a Poisson. This one might verify experimentally if we had a notion of the meaning of "galls with zero larvae". The expression between parentheses sounds nonsensical and appears as a contradictio in terminis, since galls are only formed as a result of eelworm invasion. But actually the concept is not so silly as it seems at first sight if one considers the following.

Let m be the number of true galls on the roots of a host plant (the root system of which occupies V) infested with the classes of galls, g_i (i.e. i = 0, 1, 2, 3, ... eelworms per "gall"), then we will have

$$m = \sum_{i=1}^{s} f_i \qquad (1)$$

in which the f_i are the frequencies. If further M denotes the total number of "galls" (or rather root units that could possibly be infected), we find

$$M = \sum_{i=0}^{s} f_i \qquad (2)$$

Substraction of (1) from (2) yields then

$$M - m = f_0 \qquad (3)$$

Let now q be the mean of the assumed Poisson distribution, then we define

$$q = \frac{\sum_{i=0}^{s} f_i g_i}{M} \qquad (4)$$

and the probability distribution of g_i would be

$$P(g_i) = e^{-q} \sum_{i=0}^{s} \frac{q^i}{i!} \qquad (5)$$

The frequency distribution is then

$$F(g_i) = Me^{-q} \sum_{i=0}^{s} \frac{q^i}{i!} \qquad (6)$$

so that

$$f_0 = Me^{-q} \qquad (7)$$

Substituting (7) into (3) gives:

$$M - m = Me^{-q} \quad \text{or} \quad \frac{m}{M} = (1 - e^{-q}) \qquad (8)$$

The quotient $\frac{m}{M}$ is the proportion of root units infested and this is a magnitude that can be estimated when expressed in terms of root damage.

From (8) we derive

$$q = -\ln(1 - m/M) \qquad (9)$$

Since we assumed $F(g_i)$ to be representative of the eelworm distribution, q should give us an estimate of the mean of the eelworm population in V. Equation (9), therefore, gives us the primary relation between eelworm population and root damage.

When x_1 is the observed root damage, two problems remain to be solved, in order to arrive at a law of distribution for root damage.

In answering the first question we have to admit that the relation between $\frac{m}{M}$ and x is rather vague, as in practice $\frac{m}{M}$ is not measured but estimated. This implies the introduction of a personal factor of judgement as well as some relation between factual phenomena and sensory impression. However, in the law of Weber-Fechner we find an expression of such relation, so that, when we put

$$q = ax^b \tag{10}$$

we have

$$\log q = \log a + b \log x \tag{11}$$

and finally by virtue of (9)

$$e^{-ax^b} = 1 - m/M \tag{12}$$

In equation (11) which may be considered as the secondary relation between eelworm population and root damage, more specifically estimated root damage - the regression coefficient, b, will represent the "personal factor of judgement" and log a the "threshhold of sensory stimulation or impression" (= the minimum infection detectable).

Regarding q (the mean of the Poisson distribution of larvae in the galls of a single plant), we have to realize that q, being directly correlated with the total capacity of the root system of the host and therefore indirectly with the volume of soil (V) occupied by that root system, will increase or decrease with V. In other words: taking all the plants of a seedbed together, we must expect the eelworm distribution in the galls of this association of plants to be of a different type than the distribution of eelworms in the root knots of a single plant, as a result of the distribution of q. Instances of this kind have been extensively investigated and very often the observed frequencies coincide duly with the expected values from a negative point binomial (Greenwood & Yule, 1920). This

probability distribution is based on the assumption that the distribution of the means of the different constituting Poisson series in the individuals of the association follows a Pearson type III or gamma distribution *).

Thus the presumption of a similar distribution of q in the case at hand seemed profitable. Omitting the introduction of unnecessary new and unknown parameters, I considered the Pearson type X, or exponential distribution, which actually is a special case of the type III.

Contemplating now the integral

$$P(q) = k \int_a^\infty e^{-kq} dq \qquad (13)$$

we will have to analyse the denotation of k. It will be seen from (13) that when q is zero p(q) = k, which shows that k has no physical meaning, but may be defined as the probability of q being infinitesimal. Obviously too, k is the reciprocal of the mean of (13).

From expression (10) we derive

$$dq = abx^{b-1}dx \qquad (14)$$

which on substitution into (13) yields the probability distribution of x

$$P(x_1) = k \int_a^\infty abx^{b-1}e^{-kax^b}dx \qquad (15)$$

and finally the frequency distribution of observed root damage in n (the number of plants infested) will be

$$F(x_1) = n \int_a^\infty kabx^{b-1}e^{-kax^b}dx \qquad (16)$$

Simplifying by putting ka = c, equation (16) will take the form

$$F(x_1) = n \int_o^\infty cbx^{b-1}e^{-cx^b}dx \qquad (17)$$

---

*) Kendall (1947), however, remarks without further explanation, that "there are theoretical reasons justifying this assumption".

with the understanding that, when x is expressed in percentage of root damage

$$\int_a^{\infty} cbx^{b-1}e^{-cx^b}dx = \int_o^{\infty} cbx^{b-1}e^{-cx^b}dx \qquad (18)$$

This result (18) appeared promising since Mr. H.S. Sichel, to whom I previously showed the data of an extensive field experiment, empirically fitted the ogive of my observations to the expected values of

$$y = ne^{-px^{1/q}} \qquad (19)$$

which on appropriate rearrangement of the constants is identical to the integrated formula (17). I therefore adopted in the following tests an adequate method of ogive fitting, as shown by Mr. Sichel, based on the principle of his "method of frequency moments" (Sichel, 1947), which is explicated in the appendix.

For the seedbed experiment tables V to XXXIV below give (together with the χ^2 test of significance) a comparison between the observed frequencies of table II and the expected values from the ogive

$$y = ne^{-cx^b} \qquad (20)$$

Table V: treatment I_1

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	867					
		115.9	102	13.9	$\frac{193.21}{115.9}$	1.67
0.5	751.1					
		380.6	392	11.4	$\frac{129.96}{380.6}$	0.34
1.5	370.5					
		246.4	257	10.6	$\frac{112.36}{246.4}$	0.46
2.5	124.1					
		93.8	91	2.8	$\frac{7.84}{93.8}$	0.08
3.5	30.3					
		24.7	19	5.7	$\frac{32.49}{24.7}$	1.32
4.5	5.6					
		4.8	3	.		
5.5	0.8					
		0.7	3	0.4	$\frac{0.16}{5.6}$	0.03
6.5	0.1					
		0.1	0	.		
7.5	0.0					
		867.0	867			

$\chi^2_5 = 3.90$ P = 0.28

Table VI: treatment I_2

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	242					
		71.6	69	2.6	$\frac{6.76}{71.6}$	0.09
0.5	170.4					
		106.3	106	0.3	$\frac{0.09}{106.3}$	0.00
1.5	64.1					
		43.5	45	1.5	$\frac{2.25}{43.5}$	0.05
2.5	20.6					
		14.7	19	.		
3.5	5.9			.		
		4.3	2	.		
4.5	1.6			.		
		1.2	0	1.4	$\frac{0.96}{20.6}$	0.10
5.5	0.4			.		
		0.3	1	.		
6.5	0.1			.		
		0.1	0	.		
7.5	0.0					
		242.0	242		χ_1^2 = 0.24	P = 0.64

Table VII: treatment I_3

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	199					
		63.5	65	1.5	$\frac{2.25}{63.5}$	0.04
0.5	135.5					
		78.5	76	2.5	$\frac{6.25}{78.5}$	0.08
1.5	57.0					
		34.2	32	2.2	$\frac{4.84}{34.2}$	0.14
2.5	22.8					
		13.9	18	4.1	$\frac{16.81}{13.9}$	1.21
3.5	8.9			.		
		5.5	7	.		
4.5	3.4			.		
		2.1	0	.		
5.5	1.3			.		
		0.8	0	0.9	$\frac{0.81}{8.9}$	0.09
6.5	0.5			.		
		0.3	0	.		
7.5	0.2			.		
		0.1	1	.		
8.5	0.1			.		
		0.1	0	.		
9.5	0.0					
		199.0	199		χ_2^2 = 1.56	P = 0.47

Table VIII: treatment I_4

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	245					
		67.6	57	10.6	$\frac{112.36}{67.6}$	1.66
0.5	177.4					
		102.9	125	22.1	$\frac{488.41}{102.9}$	4.75
1.5	74.5					
		46.9	31	15.9	$\frac{252.81}{46.9}$	5.39
2.5	27.6					
		18.2	17	1.2	$\frac{1.44}{18.2}$	0.08
3.5	9.4					
		6.4	11	.		
4.5	3.0			.		
		2.1	3	.		
5.5	0.9			.		
		0.6	1	5.6	$\frac{31.36}{9.4}$	3.34
6.5	0.3			.		
		0.2	0	.		
7.5	0.1			.		
		0.1	0	.		
8.5	0.0					
		245.0	245		$\chi^2_2 = 15.22$	$P < 0.001$

Table IX: treatment I_5

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	240					
		111.2	121	9.8	$\frac{96.04}{111.2}$	0.86
0.5	128.8					
		99.1	82	17.1	$\frac{292.41}{99.1}$	
1.5	29.7			.		
		23.6	37	.		
2.5	6.1			.		
		4.9	0	7.3	$\frac{53.29}{29.7}$	1.79
3.5	1.2			.		
		1.0	0	.		
4.5	0.2			.		
		0.2	0	.		
5.5	0.0					
		240.0	240		$\chi^2_0 = 5.60$	no criterion

Table X: treatment II_1

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{diff^2}{exp.f_1}$	contrib. to χ^2
0.0	40	10.9	10	0.9	$\frac{0.81}{10.9}$	0.07
0.5	29.1	14.5	16	1.5	$\frac{2.25}{14.5}$	0.16
1.5	14.6	7.5	9	1.5	$\frac{2.25}{7.5}$	0.30
2.5	7.1	3.7	2	.		
3.5	3.4	1.8	2	.		
4.5	1.6	0.8	0	2.1	$\frac{4.41}{7.1}$	0.62
5.5	0.8	0.4	0	.		
6.5	0.4	0.2	0	.		
7.5	0.2	0.1	1	.		
8.5	0.1	0.1	0	.		
9.5	0.0	40.0	40		$\chi_1^2 = 1.15$	$P = 0.29$

Table XI: treatment II_2

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{diff^2}{exp.f_1}$	contrib. to χ^2
0.0	123	30.5	28	2.5	$\frac{6.25}{30.5}$	0.20
0.5	92.5	50.3	54	3.7	$\frac{13.69}{50.3}$	0.27
1.5	42.2	25.2	24	1.2	$\frac{1.44}{25.2}$	0.57
2.5	17.0	10.7	12	1.3	$\frac{1.69}{10.7}$	0.16
3.5	6.3	4.1	2	.		
4.5	2.2	1.5	1	1.3	$\frac{1.69}{6.3}$	0.27
5.5	0.7	0.5	0	.		
6.5	0.2	0.1	2	.		
7.5	0.1	0.1	0	.		
8.5	0.0	123.0	123		$\chi_2^2 = 1.47$	$P = 0.49$

Table XII: treatment II_3

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{diff^2}{exp.\ f_i}$	contrib. to χ^2
0.0	120					
		27.6	29	1.4	$\frac{1.96}{27.6}$	0.07
0.5	92.4					
		51.9	48	3.9	$\frac{15.21}{51.9}$	0.29
1.5	40.5					
		25.9	22	3.9	$\frac{15.21}{25.9}$	0.59
2.5	14.6					
		10.0	18	.		
3.5	4.6					
		3.3	3	.		
4.5	1.3					
		1.0	0	6.4	$\frac{40.96}{14.6}$	2.81
5.5	0.3					
		0.2	0	.		
6.5	0.1					
		0.1	0	.		
7.5	0.0					
		120.0	120			

$\chi_1^2 = 3.76$ $P = 0.05$

Table XIII: treatment II_4

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{diff^2}{exp.\ f_i}$	contrib. to χ^2
0.0	88					
		29.7	29	0.7	$\frac{0.49}{29.7}$	0.02
0.5	58.3					
		44.0	44	0.0	-	0.00
1.5	14.3					
		12.0	12	.		
2.5	2.3					
		2.0	3	0.7	$\frac{0.49}{14.3}$	0.03
3.5	0.3					
		0.3	0	.		
4.5	0.0					
		88.0	88			

$\chi_0^2 = 0.05$ no criterion

Table XIV: treatment II_5

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{diff^2}{exp.\ f_i}$	contrib. to χ^2
0.0	27					
		5.4	4	1.4	$\frac{1.96}{5.4}$	0.36
0.5	21.6					
		12.6	15	2.4	$\frac{5.76}{12.6}$	0.46
1.5	9.0					
		6.4	6	.		
2.5	2.6					
		2.0	1	1.0	$\frac{1.00}{9.0}$	0.11
3.5	0.6					
		0.5	0	.		
4.5	0.1					
		0.1	1	.		
5.5	0.0					
		27.0	27			

$\chi_0^2 = 0.93$ no criterion

Table XV: treatment III_1

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	15					
		7.7	8	0.3	$\frac{0.09}{7.7}$	0.01
0.5	7.3			.		
		6.6	6	.		
1.5	0.7			.		
		0.7	1	0.3	$\frac{0.09}{7.3}$	0.01
2.5	0.0	—	—			—
		15.0	15		$\chi_0^2 = 0.02$	no criterion

Table XVI: treatment III_2

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	158					
		46.8	43	3.8	$\frac{14.44}{46.8}$	0.31
0.5	111.2					
		90.3	95	4.7	$\frac{22.09}{90.3}$	0.24
1.5	20.9			.		
		19.2	15	.		
2.5	1.7			.		
		1.6	4	0.9	$\frac{0.81}{20.9}$	0.04
3.5	0.1			.		
		0.1	1	.		
4.5	0.0	—	—			—
		158.0	158		$\chi_0^2 = 0.59$	no criterion

Table XVII: treatment III_3

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	108					
		43	43	0.0	-	0.00
0.5	65.0				$\frac{0.64}{48.2}$	
		48.2	49	0.8		0.01
1.5	16.8			.		
		13.2	8	.		
2.5	3.6			.	$\frac{0.64}{16.8}$	
		2.9	7	0.8		0.04
3.5	0.7			.		
		0.6	1	.		
4.5	0.1			.		
		0.1	0	.		
5.5	0.0	—	—			—
		108.0	108		$\chi_0^2 = 0.05$	no criterion

Table XVIII: treatment III_4

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	114					
		47.7	50	2.3	$\frac{5.29}{47.7}$	0.11
0.5	66.3					
		51.2	52	0.8	$\frac{0.64}{51.2}$	0.01
1.5	15.1					
		12.1	7	.		
2.5	3.0			.		
		2.4	2	.		
3.5	0.6			3.1	$\frac{9.61}{15.1}$	0.64
		0.5	2	.		
4.5	0.1			.		
		0.1	1	.		
5.5	0.0					
		114.0	114		$\chi_0^2 = 0.76$	no criterion

Table XIX: treatment III_5

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	32				0.01	
		7.9	8	0.1	$\frac{7.9}{}$	0.00
0.5	24.1				1.21	
		16.1	15	1.1	$\frac{16.1}{}$	0.08
1.5	8.0			.		
		6.3	9	.		
2.5	1.7			.	1.00	
		1.4	0	1.0	$\frac{8.0}{}$	0.13
3.5	0.3			.		
		0.3	0	.		
4.5	0.0					
		32.0	32		$\chi_0^2 = 0.21$	no criterion

Table XX: treatment IV_1

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	347				23.04	
		65.8	61	4.8	65.8	0.35
0.5	281.2				36.00	
		137.0	143	6.0	137.0	0.26
1.5	144.2				176.89	
		81.3	68	13.3	81.3	2.18
2.5	62.9				246.49	
		38.3	54	15.7	38.3	6.44
3.5	24.6				14.44	
		15.8	12	3.8	15.8	0.91
4.5	8.8					
		5.8	5	.		
5.5	3.0					
		2.?	2	.		
6.5	0.9					
		0.6	1	.	0.04	
7.5	0.3			0.2	8.8	0.01
		0.1	1	.		
8.5	0.2					
		0.1	0	.		
9.5	0.1					
		0.1	0	.		
10.5	0.0					
		347.0	347		$\chi_3^2 = 10.15$	P = 0.02

Table XXI: treatment IV_2

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	507				86.49	
		125.3	116	9.3	125.3	0.69
0.5	381.7				125.44	
		230.8	242	11.2	230.8	0.54
1.5	150.9				16.00	
		104.0	100	4.0	104.0	0.15
2.5	46.9				6.25	
		34.5	37	2.5	34.5	0.18
3.5	12.4					
		9.5	8	.		
4.5	2.9					
		2.3	3	.	0.16	
5.5	0.6			0.4	12.4	0.01
		0.5	1	.		
6.5	0.1					
		0.1	0	.		
7.5	0.0					
		507.0	507		$\chi_2^2 = 1.57$	P = 0.46

Table XXII: treatment IV_3

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	420					
		108.1	97	11.1	$\frac{123.21}{108.1}$	1.14
0.5	311.9					
		182.7	201	18.3	$\frac{334.89}{182.7}$	1.83
1.5	129.2					
		84.3	76	8.3	$\frac{68.89}{84.3}$	0.82
2.5	44.9					
		31.0	30	1.0	$\frac{1.00}{31.0}$	0.03
3.5	13.9					
		10.0	9	.		
4.5	3.9					
		2.9	5	.		
5.5	1.0					
		0.7	2	2.4	$\frac{4.41}{13.6}$	0.32
6.5	0.3					
		0.2	0	.		
7.5	0.1					
		0.1	0	.		
8.5	0.0					
		420.0	420			

$\chi^2_2 = 4.14$ $P = 0.13$

Table XXIII: treatment IV_4

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	271					
		58.4	46	12.4	$\frac{153.76}{58.4}$	2.63
0.5	212.6					
		111.7	135	23.3	$\frac{542.89}{111.7}$	4.86
1.5	100.9					
		60.2	38	22.2	$\frac{492.84}{60.2}$	8.19
2.5	40.7					
		26.0	34	8.0	$\frac{64.00}{26.0}$	2.46
3.5	14.7					
		9.8	8	.		
4.5	4.9					
		3.4	9	.		
5.5	1.5					
		1.1	1	3.3	$\frac{10.89}{14.7}$	0.74
6.5	0.4					
		0.3	0	.		
7.5	0.1					
		0.1	0	.		
8.5	0.0					
		271.0	271			

$\chi^2_2 = 18.88$ $P < 0.001$

Table XXIV: treatment IV_5

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{diff^2}{exp.f_i}$	contrib. to χ^2
0.0	425					
		154.0	148	6.0	$\frac{36.00}{154.0}$	0.23
0.5	271.0					
		207.4	218	10.6	$\frac{112.36}{207.4}$	0.54
1.5	63.6					
		53.2	45	8.2	$\frac{67.24}{53.2}$	1.26
2.5	10.4					
		9.1	9	.		
3.5	1.3					
		1.2	3	3.6	$\frac{12.96}{10.4}$	1.25
4.5	0.1					
		0.1	2	.		
5.5	0.0					
		425.0	425			

$\chi_1^2 = 3.28$ P = 0.07

Table XXV: treatment V_1

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{diff^2}{exp.f_i}$	contrib. to χ^2
0.0	702					
		80.8	73	7.8	$\frac{60.84}{80.8}$	0.75
0.5	621.2					
		251.9	258	6.1	$\frac{37.21}{251.9}$	0.15
1.5	369.3					
		194.4	210	15.6	$\frac{243.36}{194.4}$	1.25
2.5	174.9					
		105.3	97	8.3	$\frac{68.89}{105.3}$	0.65
3.5	69.6					
		45.6	37	8.6	$\frac{73.96}{45.6}$	1.62
4.5	24.0					
		16.8	18	1.2	$\frac{1.44}{16.8}$	0.09
5.5	7.2					
		5.0	4	.		
6.5	2.2					
		1.7	2	.		
7.5	0.5			1.8	$\frac{3.24}{7.2}$	0.45
		0.4	3	.		
8.5	0.1					
		0.1	0	.		
9.5	0.0					
		702.0	702			

$\chi_4^2 = 4.96$ P = 0.29

Table XXVI: treatment V_2

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	79					
		22.6	20	2.6	$\frac{6.76}{22.6}$	0.30
0.5	56.4					
		35.1	40	4.9	$\frac{24.01}{35.1}$	0.68
1.5	21.3					
		14.6	10	4.6	$\frac{21.16}{14.6}$	1.45
2.5	6.7					
		4.8	7	.		
3.5	1.9			.		
		1.4	1	.		
4.5	0.5			2.3	$\frac{5.29}{6.7}$	0.79
		0.4	0	.		
5.5	0.1			.		
		0.1	1	.		
6.5	0.0					
		79.0	79		$\chi^2_1 = 3.22$	P = 0.08

Table XXVII: treatment V_3

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	699					
		200.1	191	9.1	$\frac{82.81}{200.1}$	0.41
0.5	498.9					
		305.3	317	11.7	$\frac{136.89}{305.3}$	0.45
1.5	193.6					
		129.6	126	3.6	$\frac{12.96}{129.6}$	0.10
2.5	64.0					
		44.9	48	3.1	$\frac{9.61}{44.9}$	0.21
3.5	19.1					
		13.8	10	3.8	$\frac{14.44}{13.8}$	1.05
4.5	5.3					
		3.9	2	.		
5.5	1.4			.		
		1.1	4	.		
6.5	0.3			1.6	$\frac{2.56}{5.3}$	0.48
		0.2	1	.		
7.5	0.1			.		
		0.1	0	.		
8.5	0.0					
		699.0	699		$\chi^2_3 = 2.70$	P = 0.44

Table XXVIII: treatment V_4

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	358					
		44.7	41	3.7	$\frac{13.69}{44.7}$	0.31
0.5	313.3					
		143.8	147	3.2	$\frac{10.24}{143.8}$	0.07
1.5	169.5					
		101.8	92	9.8	$\frac{96.04}{101.8}$	0.94
2.5	67.7					
		46.4	60	13.6	$\frac{184.96}{46.4}$	3.99
3.5	21.3					
		15.9	13	2.9	$\frac{8.41}{15.9}$	0.53
4.5	5.4					
		4.3	3	.		
5.5	1.1			.		
		0.9	2	0.4	$\frac{0.16}{5.4}$	0.03
6.5	0.2			.		
		0.2	0	.		
7.5	0.0					
		358.0	358		$\chi_3^2 = 5.87$	P = 0.12

Table XXIX: treatment V_5

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	251					
		41.9	34	7.9	$\frac{62.41}{41.9}$	1.49
0.5	209.1					
		97.8	110	12.2	$\frac{148.84}{97.8}$	1.52
1.5	111.3					
		62.0	57	5.0	$\frac{25.00}{62.0}$	0.40
2.5	49.3					
		30.1	30	0.1	$\frac{0.01}{30.1}$	0.00
3.5	19.2					
		12.5	12	0.5	$\frac{0.25}{12.5}$	0.02
4.5	6.7					
		4.5	5	.		
5.5	2.8			.		
		1.6	3	.	$\frac{1.69}{6.7}$	0.25
6.5	0.6			1.3		
		0.4	0	.		
7.5	0.2			.		
		0.2	0	.		
8.5	0.0					
		251.0	251		$\chi_3^2 = 3.68$	P = 0.30

Table XXX: treatment VI_1

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	92					
		24.1	22	2.1	$\frac{4.41}{24.1}$	0.18
0.5	67.9					
		37.2	41	3.8	$\frac{14.44}{37.2}$	0.39
1.5	30.7					
		18.2	14	4.2	$\frac{17.64}{18.2}$	0.97
2.5	12.5					
		7.7	10	.		
3.5	4.8			.		
		3.1	3	.		
4.5	1.7			.		
		1.1	1	.		
5.5	0.6			2.5	$\frac{6.25}{12.5}$	0.50
		0.4	1	.		
6.5	0.2			.		
		0.1	0	.		
7.5	0.1			.		
		0.1	0	.		
8.5	0.0					
		92.0	92			

$\chi_1^2 = 2.04$ $P = 0.16$

Table XXXI: treatment VI_2

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	608					
		115.6	95	20.6	$\frac{424.36}{115.6}$	3.67
0.5	492.4					
		305.7	333	27.3	$\frac{745.29}{305.7}$	2.44
1.5	186.7					
		142.8	133	9.8	$\frac{96.04}{142.8}$	0.67
2.5	43.9					
		36.8	40	3.2	$\frac{10.24}{36.8}$	0.28
3.5	7.1					
		6.3	5	.		
4.5	0.8					
		0.7	1	0.1	$\frac{0.01}{7.1}$	0.00
5.5	0.1					
		0.1	1	.		
6.5	0.0					
		608.0	608			

$\chi_2^2 = 7.06$ $P = 0.03$

Table XXXII: treatment VI_3

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	228					
		52.1	43	9.1	$\frac{82.81}{52.1}$	1.59
0.5	175.9					
		92.1	109	16.9	$\frac{285.61}{92.1}$	3.10
1.5	83.8					
		48.8	35	13.8	$\frac{190.44}{48.8}$	3.90
2.5	35.0					
		21.6	25	3.4	$\frac{11.56}{21.6}$	0.54
3.5	13.4			.		
		8.6	12	.		
4.5	4.8			.		
		3.1	3	.		
5.5	1.7			.		
		1.2	0	2.6	$\frac{6.76}{13.4}$	0.50
6.5	0.5			.		
		0.3	0	.		
7.5	0.2			.		
		0.2	1	.		
8.5	0.0					
		228.0	228		$\chi_2^2 = 9.63$	$P < 0.01$

Table XXXIII: treatment VI_4

x_1	y_1	calc. f_1	obs. f_1	diff.	$\frac{\text{diff}^2}{\text{exp.}f_1}$	contrib. to χ^2
0.0	329					
		51.0	32	19.0	$\frac{361.00}{51.0}$	7.08
0.5	278.0					
		119.4	149	29.6	$\frac{876.16}{119.4}$	7.34
1.5	158.6					
		81.0	85	4.0	$\frac{16.00}{81.0}$	0.20
2.5	77.6					
		43.4	33	10.4	$\frac{108.16}{43.4}$	2.49
3.5	34.2					
		20.3	13	7.3	$\frac{53.29}{20.3}$	2.48
4.5	13.9					
		8.7	6	2.7	$\frac{7.29}{8.7}$	0.84
5.5	5.2			.		
		3.3	5	.		
6.5	1.9			.		
		1.3	2	.		
7.5	0.6			5.8	$\frac{33.64}{5.2}$	6.47
		0.4	4	.		
8.5	0.2			.		
		0.2	0	.		
9.5	0.0					
		329.0	329		$\chi_4^2 = 26.90$	$P < 0.001$

Table XXXIV: treatment VI_5

x_i	y_i	calc. f_i	obs. f_i	diff.	$\frac{\text{diff}^2}{\text{exp.}f_i}$	contrib. to χ^2
0.0	297					
		44.5	48	3.5	$\frac{12.25}{44.5}$	0.28
0.5	252.5				$\frac{144.00}{134.0}$	
		134.0	122	12.0		1.07
1.5	118.5				$\frac{129.96}{80.6}$	
		80.6	92	11.4		1.61
2.5	37.9				$\frac{1.00}{29.0}$	
		29.0	30	1.0		0.03
3.5	8.9					
		7.3	5			
4.5	1.6				$\frac{15.21}{7.3}$	
		1.4	0	3.9		2.08
5.5	0.2					
		0.2	0			
6.5	0.0					
		297.0	297			

$\chi^2_2 = 5.07$ $P = 0.08$

In the criteria above the subscript to χ^2 denotes the degrees of freedom for entry in a table of χ^2. In all cases 3 degrees of freedom had to be substracted from the number of comparable classes, as three constraints are imposed by the parameters of the distribution, viz. one for adding up to n of the expected frequencies and one for each of the constants computed, in case a and b respectively. The limit of significance has been conventionally taken at the 5 % level of P, i.e. at lower levels of P our hypothesis is rejected, at higher ones accepted as significantly in agreement with the experience.

Inspection of tables V - XXXIV shows that out of 30 distributions, 8 left no criterion by means of χ^2, namely I_5, II_4, II_5 III_1, III_2, III_3, III_4 and III_5; though it will be seen that at least II_4, III_1, III_2, III_3 and III_5 gave a very good fit. Nevertheless we will disregard all eight of them in the discussion below.

Of the remaining 22 distributions, then, another 6 (I_4, IV_1, IV_4, VI_2, VI_3 and VI_4) had to be discarded on account of the values of χ^2, so that, eventually, in 16 cases the observed frequencies were significantly coincident with the expected values from our distribution law for root damage.

This result is very satisfactory, especially on

consideration of the import of the χ^2 criterion. One should realize that the following factors are of influence on the goodness of fit: a) "chance" (P); b) sampling and c) hypothesis. A priori 5 % (= 1.1 out of 22) of the fits may be expected "bad" as a result of "chance". Since more than these were found to be rejectable, one may argue whether this is due to inadequateness of the hypothesis or to bad sampling in the cases concerned. Unfortunately there is no statistical means of judgement on this point, but a plausible decision will soon emanate from inspection of the data.

If the last columns of table VIII, XX, XXIII, XXXI, XXXII and XXXIII are scrutinized, the classes which yield rather high contributions to χ^2 can easily be detected and it will thus be seen that no doubt is left regarding the result to bad sampling. Obviously no probability law will fit the sharp peaks in the empirical distributions here at hand.

One should not wonder at the high contributions of bad sampling when it is remembered that the grading was not based on measurement but on judgement or estimates, and to this point only the experienced field worker on nematode control will fully appreciate the difficulties encountered in practice. In fact, a good deal of skill has to be acquired before successful grading can be performed.

Having obtained sufficient confidence in the validity of our hypothesis, we may now return to the question of nematode indices.

According to the above analysis, the order of magnitude of an eelworm population (E) is evidently found as $n \times m_1$ (or n times the mean), so that, in terms of the integral (17), we have

$$E = n \int_0^\infty cbx^b e^{-cx^b} dx = \frac{n\Gamma(1/b+1)}{c^{1/b}} \qquad (21)$$

which, in Bichel's notation, is equal to the first frequency moment, J_1, of the curve $y = ne^{-c \cdot t^b}$ (see Appendix).

A nematode index, as we have seen before, implies no more than a relative figure, which, naturally, should be reliable and applicable under all circumstances of comparison in the analysis of control experiments.

In this respect, apparently, J_1 offers the correct expression. Though theoretically sound, however, objections may be raised against J_1 as a nematode index. First of all, its computation is rather cumbersome (as it requires i.a. an adequate quadrature formula for the derivation of the necessary weights) and is based on grading, which - as every field worker knows - is an extremely time-thieving practice. Moreover, it introduces all the hazards of subjectivity and inconsistency of judgement, even with one and the same person, particularly at different times.

Led by these considerations, I was not completely satisfied with the results so far obtained.

Reconsidering the problem, it struck me that one consequence had not yet been drawn from the basic assumption (the random movement of the eelworm larvae, spreading from foci of infestation which again - at least in well cultivated soils - are found in random distribution) when my attention was drawn to a paper on the theory of host-parasite relationships by W.R. Thompson (1924), in which the author derived a simple mathematical expression for the occurrence of infection in a population of hosts as a result of random search by the members of a parasitic population in the same biocoenosis.

Thompson argues as follows: "Admettons que le nombre total d'hôtes disponibles soit = N, et que le nombre d'hôtes parasités après la déposition d'x oeufs soit = y.

Si le parasite sait choisir entre les hôtes parasités et les hôtes non parasités, et ne dépose ses oeufs que dans les individus indemnes, nous aurons constamment

$$y = x$$

Mais si le parasite ne sait pas distinguer les hôtes déjà

parasités de ceux qui ne sont pas parasités, la rapidité d'accroissement dans la population d'hôtes parasités, par rapport au nombre d'oeufs déposés, va diminuer, au fur et à mesure que le nombre d'oeufs déposés augmente, c'est-à-dire, au fur et à mesure que la proportion d'hôtes non parasités diminue.

Précisons. Lorsque, x oeufs ayant été déposés, le nombre (inconnu) d'hôtes parasités est y, il reste N - y hôtes indemnes. Plaçons-nous dans le cas où les nombres N et x sont très grands et imaginons que x prenne un accroissement Δx, grand lui aussi, mais tel que le rapport $\frac{\Delta x}{N}$ soit très petit. Dans ces conditions, les Δx nouveaux oeufs se repartiront de façon sensiblement homogène entre les y hôtes déjà parasités et les N - y hôtes intacts. L'accroissement Δy de y qui correspond à l'accroissement Δx de x sera donc donné par la formule très approchée

$$\Delta y = \Delta x \frac{N - y}{N}$$

qui conduit, entre les différentielles dx et dy à la relation

$$dy = dx \frac{N - y}{N}$$

C'est une équation différentielle qui déterminera y en fonction de x.

On en tire immédiatement

$$\frac{dy}{N - y} = \frac{dx}{N}$$

c'est-à-dire, puisque y doit s'annuler avec x *)

$$\log_e(N - y) = - \frac{x}{N} + \log_e N$$

et, enfin

$$N - y = Ne^{-x/N}$$ (e étant la base des logarithmes néperiens: e = 2,7182)

pour le nombre d'hôtes indemnes après la ponte de x oeufs; le nombre d'hôtes parasités est

$$y = N(1 - e^{-x/N})$$

---

*) As $\frac{dy}{N - y} = \frac{d(N - y)}{(N - y)}$ and $-\int \frac{d(N - y)}{(N - y)} = - \ln(N - y) + C$ and $\int \frac{dx}{N} = \frac{x}{N}$, we have when y = 0 and x = 0: $\ln(N - 0) = - \frac{0}{N} + C$ or $C = \ln N$. (H.)

Table XXXV

plot no.	x_1 = E = $-N\ln(1-n/N)$	x_2 = J_1	plot no.	x_1 = E = $-N\ln(1-n/N)$	x_2 = J_1
I_1	1558.83	1287.72	IV_1	530.71	531.08
I_2	262.52	269.60	IV_2	664.14	605.25
I_3	236.48	235.65	IV_3	530.73	514.15
I_4	316.94	299.17	IV_4	383.25	380.40
I_5	257.42	177.85	IV_5	485.13	360.18
II_1	41.32	58.25	V_1	1215.29	1273.13
II_2	130.16	163.81	V_2	81.30	88.96
II_3	131.01	156.15	V_3	854.33	800.59
II_4	97.80	77.73	V_4	638.56	580.79
II_5	33.04	34.29	V_5	434.67	401.65
III_1	15.10	8.92	VI_1	90.43	120.68
III_2	196.10	136.65	VI_2	992.28	736.97
III_3	115.62	90.39	VI_3	255.15	319.51
III_4	122.38	88.07	VI_4	509.32	573.80
III_5	33.08	34.71	VI_5	588.84	421.90
	3547.80	3118.99		8260.13	7709.04

$S(x_1) = 11807.93$ $S(x_2) = 10828.03$

$\bar{x}_1 = 393.60$ $\bar{x}_2 = 360.93$

The regression coefficient of x_2 on x_1 is given by

$$b_1 = b_{x_2x_1} = \frac{S(x_1x_2) - \bar{x}_1 S(x_2)}{S(x_1^2) - \bar{x}_1 S(x_1)} = 0.874$$

and its standard deviation

$$s_{b_1} = \sqrt{\frac{S(x_2 - \bar{x}_2)^2 - \frac{S^2(x_1 - \bar{x}_1)(x_2 - \bar{x}_2)}{S(x_1 - \bar{x}_1)^2}}{S(x_1 - \bar{x}_1)^2}} = 0.0332$$

so that fiducial limits can be set (as $t_{0.05}$ for 28 d.f. = 2.048):

$l_1 = 0.874 - 2.048 \times 0.0332 = 0.806$ and $l_2 = 0.874 + 0.068 = 0.942$

Mutatis mutandis $b_2 = 1.100 \pm 0.085$.

Les valeurs de y et de (N - y) données par cette formule ne seront exactes qu'à condition que x et N soient très grands. En d'autres termes, pour savoir si, dans un cas donné la distribution des parasites dépend uniquement du hasard, il faudra examiner un nombre considérable d'hôtes".

"Il est évident que le pourcentage de parasitisme ne dépend pas de la valeur numérique absolue de x et de N, mais seulement du rapport entre ces deux valeurs".

In a later paper (1939) Thompson expanded his theory and proved, that no difference ensues whether the parasites are able to search the entire territory of the host population or, when limited in their range, search in smaller areas if these areas are themselves distributed at random amongst the hosts.

It should be worth while, therefore, in consequence of Thompson's formula (last equation above) to estimate the relative values of eelworm populations (E) from the equation

$$E = - N \ln(1 - n/N) \qquad (22)$$

and to compare the results with the corresponding values of J_1, as arranged in table XXXV opposite.

Since the figures for x_1 (= E) and x_2 (= J_1) are pure numbers, they are directly comparable. But lacking a mathematical relation between the two, we can not derive the one from the other, nor can any preference be given statistically with respect to the reliability of either of them, unless we obtain more information about the one or the other (see page 79). All we know about x_1 and x_2 so far is that their evaluation results from the same principle assumed, so that, if our assumption and the deductions made from it are consistent, J_1 and E should be highly correlated.

The regression of x_2 on x_1 was found to be

$$b_{x_2 x_1} = 0.874 \pm 0.068$$

and the regression of x_1 on x_2 was:

$$b_{x_1x_2} = 1.100 \pm 0.085$$

through which the coefficient of correlation is determined as

$$r = \sqrt{b_{x_2x_1} \times b_{x_1x_2}} = 0.981$$

Fiducial limits (l_1 and l_2) can be set to the value of r by application of the formula *)

$$l_1 = z - ts_z$$

$$l_2 = z + ts_z$$

where $z = \frac{1}{2}\ln\frac{(1 + r)}{(1 - r)}$ and $s_z^2 = \frac{1}{n - 3}$ (n being the number of pairs compared) with t at P = 0.01.

In this way we obtain

$$l_1 = 0.948 < r = 0.981 < l_2 = 0.992$$

The correlation between x_1 and x_2 appears to be so high, that with confidence we may consider E to be identical with J_1. One could also say that x_1 and x_2 correspond in 98% of their characteristical factors. Naturally a certain percentage is sacrificed to sampling error, but evidently this percentage is very small.

It will be observed - on inspection of table XXXV - that in many cases considerable differences exist between the values of J_1 and E. This becomes especially apparent when the standard deviation of the differences is determined, which is easily done by the formula *)

$$s_d^2 = s_{x_1}^2 + s_{x_2}^2 - 2rs_{x_1}s_{x_2}$$

yielding

$$s_d = 1091.83$$

This is an extraordinary high figure which tends to upset our faith in the comparison.

Let us, therefore, analyse this point a little further. The experiment showed that, though J_1 was comparable with $S(f_1x_1)$ in a high percentage of cases (16 out of 22, or nearly 73%) on the evidence of which our confidence in J_1 was based, there

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*) See Snedecor (1946).

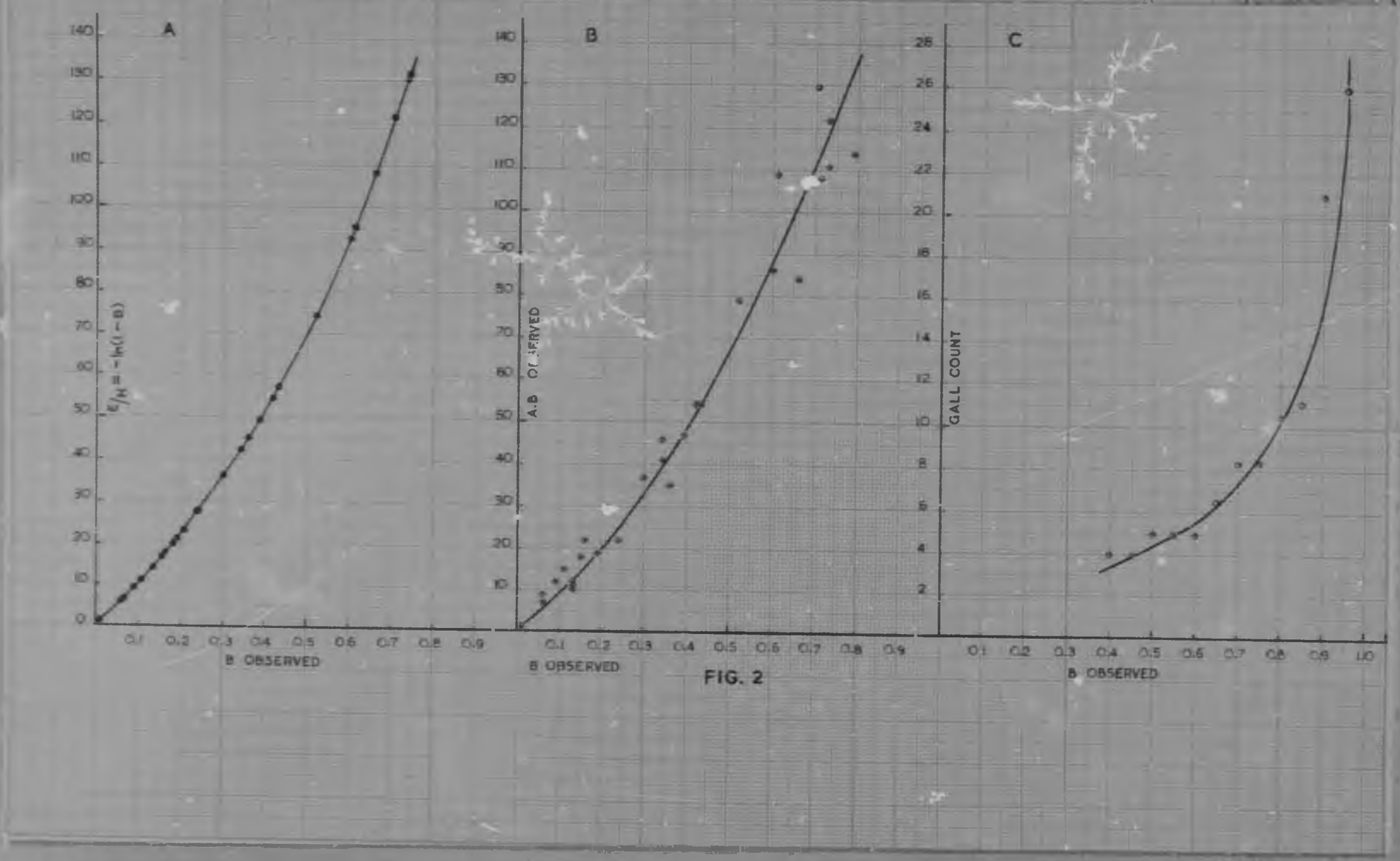
A
B
C
$\epsilon/H = -\ln(1-B)$
A.B OBSERVED
GALL COUNT
B OBSERVED
B OBSERVED
B OBSERVED

FIG. 2

still remained some 27 % of cases, that actually rejected the hypothesis, but yet were brought into consideration in the correlation table. Moreover, the 8 discarded distributions that offered no criterion, were introduced as well. When further it is remembered that both J_1 and E are approximations to the true magnitude of the eelworm population, the outcome should not create a stir. But still, one may ask the question whether it is either or both of these two estimates (J_1 and E) that is unreliable.

In reply I am inclined to ascribe the discrepancies to the inconsistency of J_1, since the theory requires for the applicability of equation (22) that N be large, and in praxis this has been observed throughout. J_1, on the other hand, is based on grading with which over- or underestimation is easily introduced. On the <u>average</u> J_1 may be a useful statistic (explaining the high correlation between J_1 and E), but in particular instances it may be a rather misleading approximation.

More information on the validity of E could be obtained from figures published by Godfrey (1934 a). Studying his data I plotted a graph of the percentage of plants (cowpeas) infested ($^n/N$) against the average number of galls per plant observed by him. This graph (fig. 2 c) showed a striking similarity with my own data (fig. 2 b) when $^n/N$ was plotted against $S(f_1x_1)/N$. Both curves betrayed a logarithmical nature which required closer inspection.

Assuming now that the average number of galls per plant, as observed by Godfrey, is just another way of expressing the value of $^E/N$ (taking for the moment: gall count = f(E) to be a rectilinear relationship), both curves will express the trend of the statistic A (the estimated average individual infestation of the parasitized plants). Fig. 2 a gives for comparison the expected curve: $\hat{A} = {}^E/n = -\frac{N}{n} \ln(1 - {}^n/N)$.

Let us examine Godfrey's gall count as a function of E.

Table XXXVI

x_1 = Σ	actual average gall counts of plots	x_2 = mean	variance
299.57	26	26	0
230.26	10 20 22 26 28	21.2	49.2
189.71	8 8 8 10 10 10 14 14 18	11.1	12.3
160.94	6 8 8 8 10 10 12 12 12 14 16	10.5	9.4
138.63	6 6 8 8 10 10 10	8.3	3.1
120.40	6 6 6 6 8 8 8 8 10 10 12 12	8.3	5.3
104.38	6 6 6 6 6 6 6 6 6 6 8 8 8	6.5	0.5
91.63	6 6 6 6 6 6 6 6 6	6.0	0
79.53	6 6	6.0	0
69.31	4 4 4 4 4 6 6 8	5.0	2.3
59.78	4 4 4	4.0	0
51.08	4 4	4.0	0
1596.14 = $S(x_1)$		$S(x_2)$ = 116.9	
133.01 = $\bar{x}_1$		$\bar{x}_2$ = 9.74	

x_2/x_1
0.087
0.092
0.059
0.065
0.060
0.069
0.062
0.065
0.075
0.072
0.067
0.078
0.071 average

The regression coefficient of x_2 on x_1 is

$b_1 = b_{x_2x_1} = 0.0886 \pm 0.017$

and the regression coefficient of x_1 on x_2

$b_2 = b_{x_1x_2} = 10.524 \pm 1.988$

(for the formulae applied in the computations see table XXXV opposite page 77)

Data from Godfrey, 1934 a.

The complete data are given in table XXXVI opposite, from which the correlation coefficient was calculated as

$$r = 0.966$$

with fiducial limits

$$l_1 = 0.823 < r = 0.966 < l_2 = 0.994$$

This correlation is remarkably high and attention is drawn to the fact that r is more or less of the same order as in the case of $r_{J_1,E}$, while the difference of E and x_2 (the gall count) is considerably larger than in the former case. But J_1 and E both estimated the eelworm population itself, whereas a gall count (though highly correlated with eelworm count, as was shown by Godfrey) a priori should be expected very much lower than the value of E, since it expresses per se only part of the population. This is readily expressed by the regression coefficients.

Computing here a variance of the difference, therefore, will be meaningless, but the data offer the possibility of analysing the regression in more detail. To this effect we have to examine the group variances (column 3 of table XXXVI) to see whether they are homogeneous. According to Bartlett's criterion of homogeneity this hypothesis had to be rejected on trial, but inspection of table XXXVI shows that no meaning can be given to the variances in actual gall counts, other than inherence to sampling. In any case the x_1 (x_1 = E) are measured practically without error and the corresponding values of y (actual gall counts) are random samples, so that the variances seem to be without any particular interest. Moreover, the ratio's x_2/x_1 (last column) are fairly consistent. One thing and another permits us to consider the regression coefficient $b_{x_2x_1}$ ($= b_{yx}$) as an unbiased estimate of the population regression coefficient β.

The regression equation is then given by

$$\hat{y} = \bar{y} + 0.0886(x - \bar{x})$$

Table XXXVII below gives the values of y (observed x_2 = mean value of individual gall counts) and $\hat{y}$ (expected value of x_2) and other necessary details.

Table XXXVII

$x_1=x_1=E$	$x - \bar{x}$	$y = x_2$	$y - \bar{y}$	$\hat{y}$	$d_{yx} = y - \hat{y}$	$d_{yx}^2 = (y - \hat{y})^2$
299.57	+166.56	26.0	+16.3	24.5	+1.5	2.25
230.26	+ 97.25	21.2	+11.5	18.4	+2.8	7.84
189.71	+ 56.70	11.1	+ 1.4	14.8	-3.7	13.69
160.94	+ 27.93	10.5	+ 0.8	12.2	-1.7	2.89
138.63	+ 5.62	8.3	- 1.4	10.2	-1.9	3.61
120.40	- 12.61	8.3	- 1.4	8.6	-0.3	0.09
104.98	- 3.03	6.5	- 3.2	7.3	-0.8	0.64
92.63	- 41.38	6.0	- 3.7	6.1	-0.1	0.01
79.85	- 53.16	6.0	- 3.7	5.0	+1.0	1.00
69.31	- 63.70	5.0	- 4.7	4.1	+0.9	0.81
59.78	- 73.23	4.0	- 5.7	3.3	+0.7	0.49
51.08	- 81.93	4.0	- 5.7	2.5	+1.5	2.25

$S(x)=1596.14$ $S(y)=116.9$ $S(d_{yx}^2) = 35.57$

$\bar{x} = 133.01$ $\bar{y} = 9.7$

The total variance of y may now be analysed as follows:

Table XXXVIII

description	symbol	degrees of freedom	sum of squares	variance
the mean	$\bar{y}$	1	$S(\bar{y}^2)=\frac{s^2(y)}{n}$ = 1138.9	1138.9
deviations determined by b and a fixed set of x_1	$\hat{y} - \bar{y} = bx$	1	$\frac{S(\hat{y} - \bar{y})^2}{S(x - \bar{x})^2}$ = 443.4	443.4
random deviation from regression	$y - \hat{y} = d_{yx}$	10	$S(d_{yx}^2)$ = 35.6	3.6
total	y	12	$S(y - \bar{y})^2$ = 1667.9	

The very small variance of d_{yx} is here of interest and makes any further examination with regard to rectilinearity of regression superfluous.

Godfrey determined a linear correlation between gall count and $^n/_N$ of 0.73 $\pm$ 0.035 and a curvilinear correlation of

0.948 ± 0.013 *). Derivation of the latter (represented by him as η) is not given in his paper, but is likely to have been found by means of some rectification formula. In any case, Godfrey had no explanation for this correlation coefficient and concludes without comment or reference to any principle: "This can be interpreted as indicating that it would be better, instead of actually counting number of galls, to classify the indicator plants only as infected and free, which can be done very quickly after the roots are thoroughly washed".

It is worth noting that Godfrey's η (though slightly lower) corresponds very well with the coefficient of correlation between gall count and E, as determined above, which - I contend - is not accidental. His remarks, however, cited above, can be justified only when seen against the background of an analysis of his data as given here, which offers a biological interpretation of the relations.

When seen in the light of these investigations, the expression $E = -N\ln(1 - n/N)$ hardly needs any further recommendation as a reliable estimate of relative eelworm populations. In practice I found that N should be not less than about 25, but preferably larger (cf. also Godfrey, 1934 a). On this condition the value of E should give a quickly determinable and accurate index figure for comparative studies, and for this reason it has been adopted throughout the statistical part of the ensuing research.

Estimates of eelworm populations by means of root damage based on one of the following formulae:

$A = \frac{S(f_1 w_1)}{n}$; $B = n/N$; $AB = \frac{(f_1 w_1)}{N}$; n (when N is constant) or $S(f_1 w_1)$ are unreliable as nematode indices.

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*) The fiducial limits are incorrectly given in this way. It is not clear how Godfrey calculated them.

SILVER AS A GERMICIDE — While charged with investigations on the suitability of constructing filters that would safely allow the utilization of contaminated river water for irrigation purposes where eelworm-susceptible crops are concerned, I came across some literature on water sterilization by means of minute dosages of silver (Brandes, 1934 a, b *); Moiseev, 1934; Just & Szniolis, 1936) which roused my interest as it seemed to open immense possibilities in new lines of research.

The method originated in Germany through the work of Dr. Georg A. Krause, in 1928, and gradually - first in Europe and later in America and elsewhere - its application spread into various branches of industry. The quantities of silver required for sterilization of liquids appeared to be so small that expenses on the metal are sufficiently reduced to render the results definitely economical. To-day not only drinking water, but also foods and beverages are treated with silver for better preservation of quality and taste (Hoffmann, 1935; Anonymus, 1938).

The germicidal properties of silver were tested in different ways and experiments soon showed that, besides bacteria, larger organisms, such as e.g. plankton (Daphnia), tadpoles, fish larvae and even goldfish ar well were highly susceptible to its toxic influence.

OLIGODYNAMIC ACTION — From the classical work of Carl von Nägeli on the effect of infinitesimal concentrations of certain metals on plants, published in 1893, originated the term "oligodynamic action" **) now generally used for the action of trace elements or oligopleronts ***).

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*) With extensive bibliography on the subject.

**) ὀλίγος (oligos) = little; δύναμις (dynamis) = power.

***) πλήρης (pleres) = sufficient. This modern word (see Koningsberger, 1947), though more descriptive than the former, has not yet found its way into the literature.

Physiologically speaking, oligodynamic action is not distinctly defined, and the effects of many substances, different from metals or other pure elements, even complicated chemical compounds would fall in the realm of oligodynamics if the concept were characterized only by the action of concentrations in an order of magnitude of, say, 10^{-5} or less.

But restricting ourselves to elements, the following were found to be of outstanding importance:

copper, zinc, manganese, molybdenium, vanadium, selenium, boron, silicon, gallium, scandium, silver, arsenic, lead, iodine, fluorine, antimony, chromium.

Both flora and fauna are concerned here, and the oligodynamic effect may be beneficial (zinc: Aspergillus niger, Viola calaminaria; molybdenium: Azotobacter chroococcum; iodine: glandula thyreoidea; fluorine: teeth; arsenic: nails and hair) or detrimental (silver, copper: Spirogyra and various Algae; silver: Staphylococcus, Bacterium coli, etc; lead, arsenic, antimony, chromium, fluorine: all cause well-known symptoms of poisoning in animals and human beings).

Concentrations of silver used for water sterilization range from 0.9 mgm to as little as 0.05 mgm per liter and even concentrations as small as 10^{-9} proved to be effective under certain conditions.

Frombertz & Heiss (1937) proved by means of simple but very elegant experiments that the silver ion is the oligodynamic agent in solutions containing that metal. They were also able to show that for the liberation of free silver ions potential differences (in other words: a certain amount of polarization) are essential in the system, which may be brought about by the introduction of a conductive unit of metallic silver and silver chloride or silver and silver hydroxide into the solution.

The threshold of oligodynamic action was determined by these authors as $2 - 3 \times 10^{-11}$ grammolecules of silver per liter, which is equal to about 2.7×10^{-12} gm Ag per cc.

Though the majority of silver salts is known to be highly insoluble, the concentration of silver ions in all such solutions - with the only exception of silver sulphide (the solubility product of Ag_2S is 1.52×10^{-49} grammolecules per liter at 18° C, giving a concentration of 7.8×10^{-25} grammolecules of Ag, which is equal to 8.42×10^{-26} gm silver per cc) - is still high enough for oligodynamic action.

Metallic silver itself has a very high "solution pressure" in water, i.e. from the oligodynamic point of view. Its solubility has been determined by Just & Szniolis (1936) as 40 gamma per liter, which is equal to 2.4×10^{14} ions of silver per cc. The metal goes very slowly into solution, unless its surface of contact with the solvent is tremendously enlarged (s.c. "Katadyn" silver) or electrical potentials are exerted.

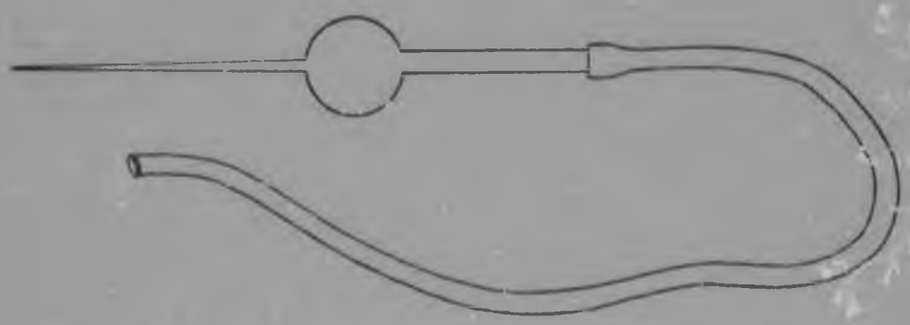
As the efficiency of the current is usually ± 50 % only, owing to loss of electricity to anions that fail to dissolve the silver at the anode, a current of 1 Ampère during one hour normaliter brings about 2.0115 gm of silver into solution.

Most of the technical applications of ionic silver sterilization are based on the principle of galvanic solution.

IONIC SILVER AS A NEW NEMATOCIDE With the above information at hand, the expectation that silver ions would also be toxic to the terricolous larvae of Heterodera marioni, seemed to be justified. In vitro I first tested out solutions with different concentrations of the highly soluble silver nitrate on the motions of newly hatched eelworms. The results were striking: concentrations as low as 1 part in 10 000 000 were found to be highly toxic. Thus the way was opened to systematic research into the possibilities of practical application (cf. Hovy, 1939).

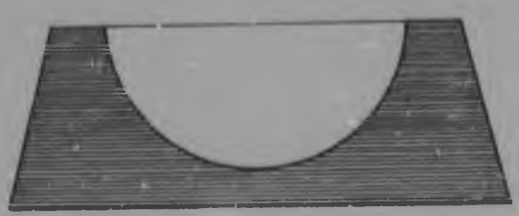
LABORATORY TECHNIQUE Heavily infested host plants were collected in tobacco lands and a number

FIG. 3



MICRO-PIPETTE FOR EELWORM

FIG. 4



EXCAVATED GLASS BLOCK

of matured root knots cut off. These were well washed and placed in Petri-dishes with distilled water. With the aid of a dissecting microscope *) some 50 oothecae were teased out by means of dissecting needles and kept in a separate dish with distilled water at room temperature. As the jelly masses contain numerous larvae in all stages of development inside the eggs, one soon obtains a considerable number of parasites ready for experimentation. All utensils have to be thoroughly washed with nitric acid and distilled water in order to eliminate even the smallest traces of silver from former experiments.

Though the eggs are able to survive for a long time (see page 25) I always used freshly hatched larvae to minimize working with possibly poisoned specimina.

A micro-pipette (fig. 3) to handle separate larvae was made in the following way. In a glass tube of 3 mm bore a little globe of about 1 cm was blown, whereafter one end was pulled out into a capillary just wide enough for a single larva to pass through. The entire pipette is not more than about 6 cm long and supplied with a thin rubber tube of at least 50 cm length.

When using this pipette by placing the free end of the rubber tubing in the mouth, one can easily pick up single nematodes out of the Petri-dish and select specially vigorous ones if desired. The little globe in the pipette is useful as it prevents the sudden sucking in of larvae and moreover, since the larvae are heavier than water, one can collect all the eelworms into the capillary by holding the pipette vertically for a while and then, by means of a little blow, expel the larvae into an experimental container without introducing too much water into the solution that has to be tested. No significant dilutions will occur in this way. A single drop of water

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*) Leitz Stand UK III was found to be a most convenient binocular for this type of work as its large table and cross motion of the tube allow the objectives to be traversed over great areas for inspection.

FIG. 5

NORMAL
MOVEMENTS

FINAL STAGE

2 – 8: EELWORMS DYING OF SILVER POISONING

9 – 11: DEATH THROUGH OTHER CAUSES

less than 1.5 cubic mm may contain a large number of larvae.

In the experiments I used excavated glass blocks (as depicted in fig. 4) containing ± 17 cc. Twelve of these will just fill out the space on the table of a [illegible] UK III binocular microscope, so that comparison of different solutions or replications of the same experiment is largely facilitated. Another advantage of these glass blocks is that, as a result of their semi-spherical excavation, the eelworms tend to collect in the centre, allowing the application of high-power magnifications with all the larvae still in the optical field so that counts can be made or their behaviour examined at a glance.

In each glass block 25 eelworms were observed. Larger numbers impede quick counting of crawling larvae, while smaller numbers tend to exaggerate the influence of individual variances in lethality.

With eelworms placed in silver solutions I noticed a remarkable trend in the way they succumb to the toxicity of the oligodynamicon. The impression is obtained as if a kind of paralysis starts from the tail, gradually proceeding craniad. It is difficult to decide whether this is a real paralysis or else a muscular rigor, although I am inclined to assume the former. In any case the normal, serpentine agitations are obviously hampered, first at the caudal end until adequate movements can no longer be performed. Fig. 5 shows various stages in this process. Sudden spasmodic contractions sometimes occur in an absolutely abnormal way (fig. 5: 3, 4, 5) and when the oesophageal nerve ring (the "central nervous system") is reached, only the head is able to move slowly (fig. 5: 7) at sharp angles from the rest of the body. The final stage is always a typical rigor mortis (fig. 5: 8) when the larva appears as a faintly curved bar. This instant I have always taken as a criterion for the worms being dead. As far as I am aware, Godfrey & Hoshino (1937) are the only authors that ever described this last stage before, which they, too, considered as an unambiguous sign of death.

The paralysing effect, described above, is characteristic of silver poisoning and has - to the best of my knowledge - never been observed before. In fig. 5 three different end stages in death through various other causes are shown. Rigor mortis, however, will almost always ensue in the described way, though often only after a long time, whereas in the case of silver poisoning it is always directly following the ceasing of movements.

The phenomena mentioned strongly suggest an infiltration of the nervous system with silver. The infallible results obtained with the classical histological technique as developed by Bielschowsky (1903, 1925) and Ramon y Cajal (1910) for staining nerve fibres by means of silver impregnation, have proved the great affinity of silver to nervous tissues, and this certainly tends to invigorate the hypothesis that the nervous system of the eelworms is first attacked by the silver ions.

As a measure of oligodynamic toxicity the time taken by the larvae to reach the final stage of rigor mortis has been correlated with the concentration of the solution. In this way comparative observations with other agents could be made as well.

THE EFFECT OF SILVER ON THE TOBACCO PLANT Twelve young tobacco plants of 5 cm height were placed in 500 cc jars filled with a physiological nutrient solution *). Two control plants received no further treatment, whereas each of the following silver concentrations (in the form of silver nitrate) was administered to two plants in addition to the nutrient solution, respectively: 1 gm, 0.1 gm, 0.01 gm, 0.001 gm and 0.0001 gm of silver per litre of water. All plants thrived equally well, except the two plants that received the

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*) The solution contained 0.8 gm $Ca(NO_3)_2$; 0.2 gm KNO_3; 0.2 gm KH_2PO_4; 0.2 gm $MgSO_4$ and a trace of $FePO_4$; aq. dest. 1000 cc. No precipitate is formed when this solution is combined with $AgNO_3$, at least not in the concentrations used here.

strongest concentration of silver nitrate, viz. containing 1 gram of silver per litre. These two plants died after 26 days, having been stunted right from the beginning of the experiment *). In 24 hours a black precipitate of metallic silver was noticed on the roots of the plants and the walls of the jars with the solutions 1.0; 0.1 and 0.01 gm Ag per litre. In the other jars no precipitate was visible. This adsorption of metallic silver is a result of photolytic dissociation of silver salts which is difficult to prevent, unless one would work in complete darkness. The results of the experiment, however, are not greatly influenced by this effect.

Green Algae started to develop in the two control jars already after 24 hours (the jars were placed in bright shaded light on a veranda), while all the jars with silver solutions did not show any trace of Algae throughout the duration of the experiment which lasted for two months.

Obviously a concentration of 1×10^{-3} of silver was not tolerated by the tobacco plants, but this was an extremely high concentration from the point of view of oligodynamic action, one that would never be practically considered.

Parallel with these water cultures ran a similar experiment with twelve tobacco plants in pots. In this case all the silver concentrations were well tolerated and no appreciable differences could be detected in the plants, nor was any silver precipitated in the leaves. Adsorption of the silver to soil particles must be responsible for these results.

In field experiments the administration of silver seemed to stimulate growth in tobacco plants, as shown by the average length (see page 108).

FURTHER EXPERIMENTS WITH SILVER To test the toxicity of metallic silver I proceeded as

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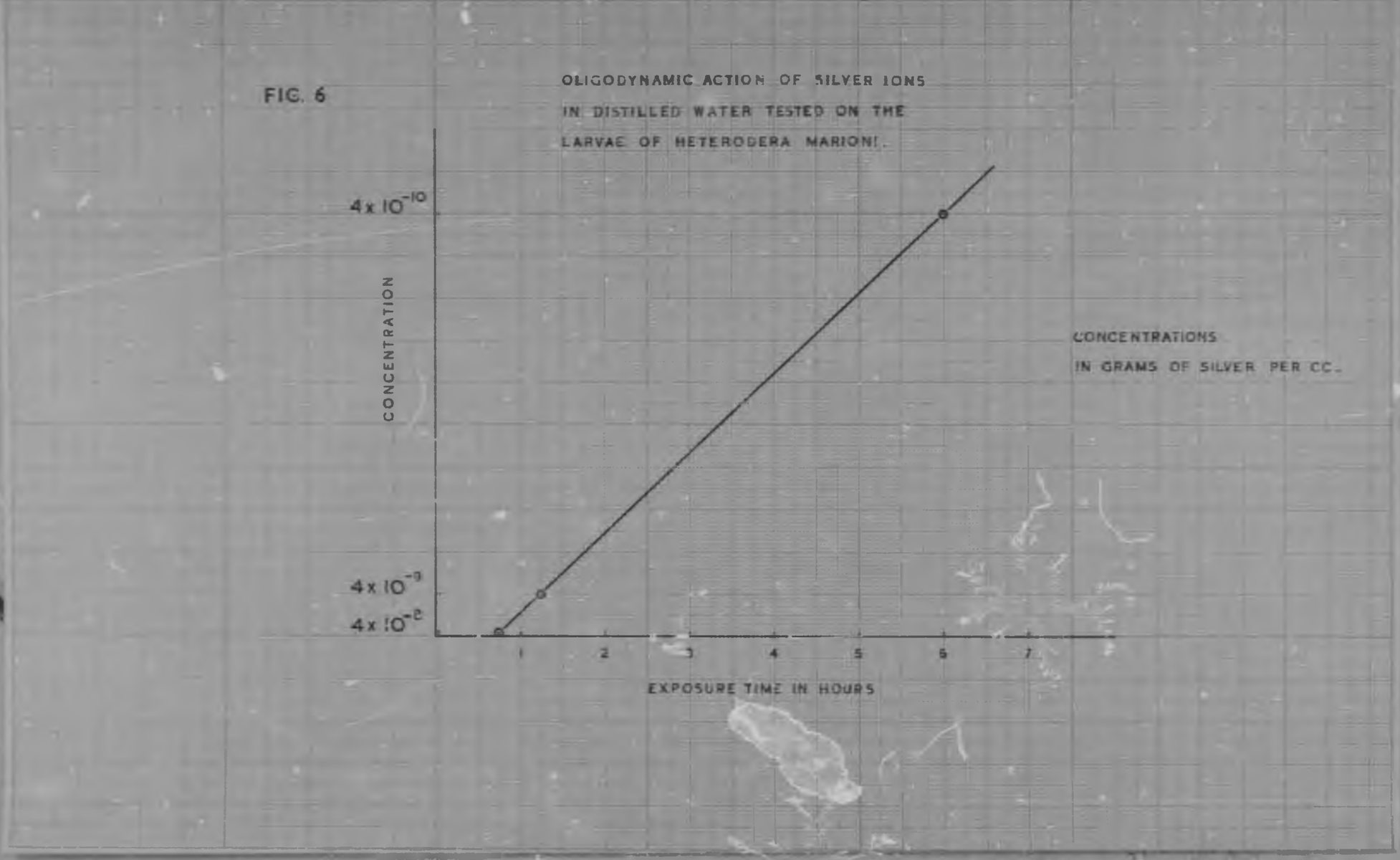
FIG. 6


OLIGODYNAMIC ACTION OF SILVER IONS
IN DISTILLED WATER TESTED ON THE
LARVAE OF HETERODERA MARIONI.
4 x 10⁻¹⁰
CONCENTRATION
4 x 10⁻⁹
4 x 10⁻⁸
1
2
3
4
5
6
7
EXPOSURE TIME IN HOURS
CONCENTRATIONS
IN GRAMS OF SILVER PER CC.

follows. In a glass flask of 500 cc a silver mirror was precipitated by means of reduction of an ammoniated solution of silver nitrate in the well-known way. After the mirror had settled, the flask was washed repeatedly and refilled with distilled water which was left standing for two days.

The presence of silver in this water could be made visible in a test tube by adding a few cc of a normal solution of potassium iodide, which resulted in the formation of a just discernable haze *). With H_2S the effect is still more pronounced. Especially when seen from above with incident light the haze can be well distinguished from a control tube with equal amounts of water and KJ solution. Ordinary titration of such minute quantities of silver is impossible and special methods are required to obtain the correct titre (Just & Szniolis, 1936).

Eelworms put in a sample of this silver solution were killed in 45 minutes. Prolonged contact of the water with the silver mirror in the flask, up to several months, did not result in a shortening of the time, necessary to kill the eelworms, so that the solution was considered saturated. Just & Szniolis (l.c.) determined the concentration of a "saturated" silver solution as 4×10^{-8} gm Ag per cc. A decuple dilution of the original "silver water" required 75 minutes for killing all the eelworms, and one hundredth of the original strength took 6 hours (see fig. 6 for the relationship between dosage and exposure time). The assumed concentration of 4×10^{-10} gm Ag per cc is well above the threshold of oligodynamic action (see page 84).

With a view of comparing the toxicity of silver with other elements, I tested solutions of one part by weight in a million of solvent made up for the following elements in simple ionic form: chlorides or nitrates of mercury, zinc, copper, selenium, lead, alluminium, antimony, lithium, cobalt potassium, sodium,

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*) The solubility product of AgJ is 1.5×10^{-16} grammolecules per litre at 25° C, so that a saturated solution of AgJ will still have a concentration of 1.32×10^{-9} gm Ag per cc.

manganese, magnesium, tin, iron, calcium, barium, bismuth and the metal-like compound ammonium (NH_4); further the potassium or sodium salts of chlorine, bromine, iodine, fluorine and the halogenous compound cyane (CN). With the sole exception of zinc, none of these elements had the slightest effect on the vitality of the eelworms after 24 hours of exposure.

The eelworms in zinc chloride were not dead, but showed signs of intoxication in that their movements were much slower than normal. I therefore subjected zinc to a closer examination and observed that 10 mgm of zinc per liter (1 part by weight in 10^5 parts of water) killed the larvae in 4 hours time. Zinc thus showed to have some detrimental influence, though by no means comparable with silver. With the latter element 3.8 parts in 10^7 parts of water sufficed to kill all eelworm larvae within 30 minutes. Nevertheless, the results with zinc were of certain interest, as they confirmed a former observation, when I noticed that eelworms did not survive in pots in which a zinc label was inserted next to a tobacco plant. The solution pressure of zinc is known to be rather high and many zinc salts are soluble.

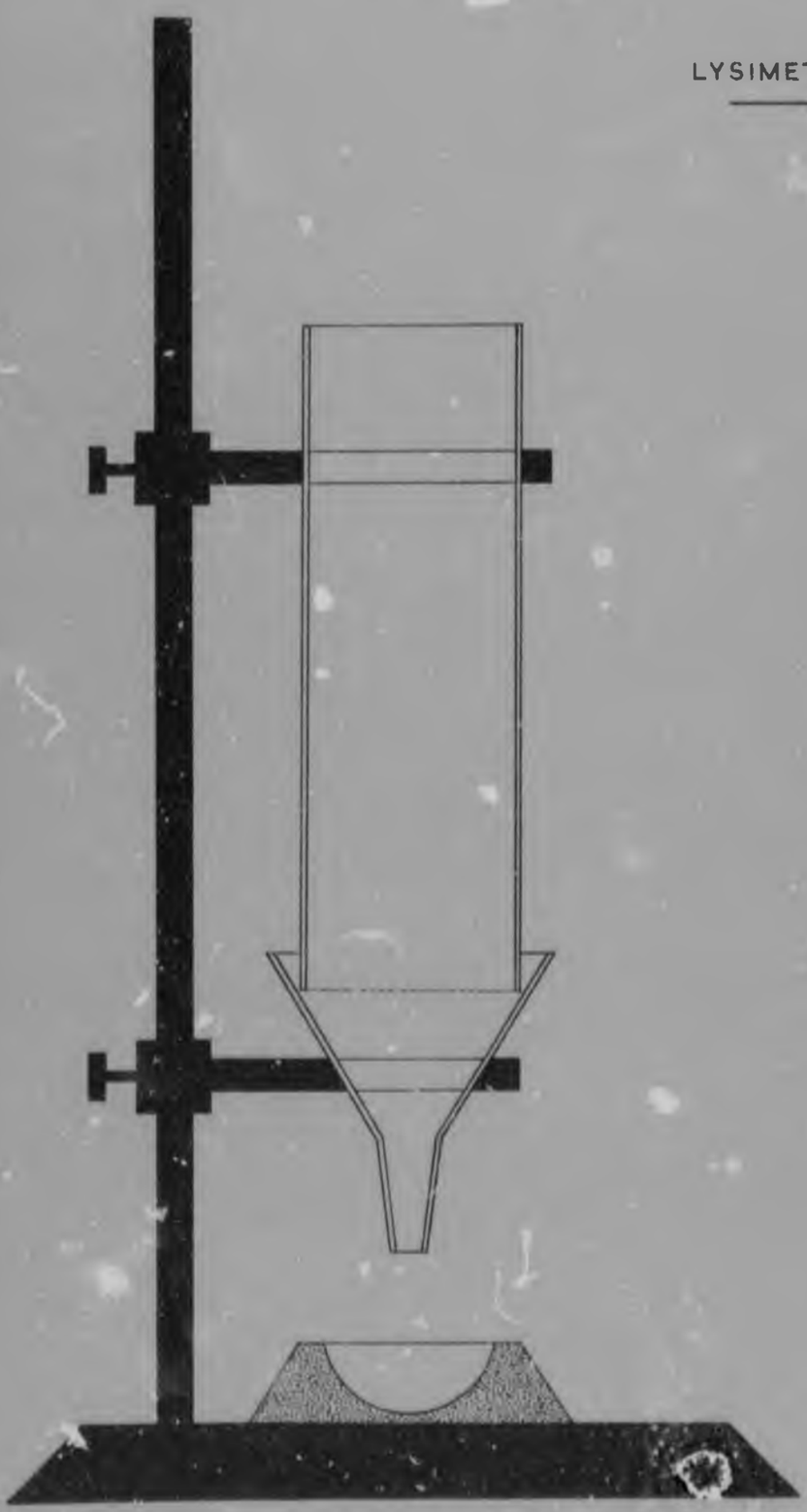
Complex silver ions were also examined. Alkali-silver compounds as $AgK(CN)_2$ and $AgNa(CN)_2$ are readily made by precipitating AgCN in a solution of $AgNO_3$ by means of KCN (or NaCN) and re-dissolving the silver cyanide in excess of alkali cyanide. Such compounds I found absolutely inactive (except in high concentrations, when a poisonous influence of the cyanide ion is exerted; see experiment 28 on page 96). This is fully in agreement with the theory that only free silver ions are oligodynamically active. Whereas silver cyanide is highly potent, due to the presence of the Ag^+ ion, the double cyanides are dissociated into, e.g., AgK^{++} (a complex silver-alkali ion) and 2 CN^- ions.

In vitro, colloidal silver has a potency similar to metallic silver.

The efficiency of silver ions as a nematocide was sufficient-

FIG. 7

LYSIMETER



ly proved by the above experiments but, since silver has the disadvantageous property of being rather quickly adsorbed on active surfaces, the question arose as to how silver compounds would behave in the soil.

To investigate this problem I applied a lysimeter as shown in fig. 7. The apparatus consists of a glass tube of 4 inches bore and 12 inches long, closed at one end with copper wire gauze and placed on a stand over a funnel.

The lysimeter was filled with 2 lbs of sun-dried, well-screened soil, whereafter 500 cc of the solution to be tested was percolated through it. The apparatus was carefully cleaned before each new experiment and refilled with fresh soil. The solutions were tested before and after going through the lysimeter. Observations were made in the way described on page 87.

The following compounds were tested: silver proteinate ("Protargol"), silver chloride, silver hydroxide, silver carbonate, metallic silver, zinc chloride, lead nitrate, copper nitrate, sodium cyanide and a few combinations as will be seen from table XXXIX where the results of these experiments have been tabulated.

Table XXXIX

exp. no	solution tested *)	lysimeter treatment	movements ceased after	rigor mortis	duration of exp.
1a	Ag-prot. 1 x 10^{-6}	none	56 min.	2 hrs	
1b	idem	sandy soil			
1c	distilled water	none			24 hrs
2a	Ag-prot. 1 x 10^{-4}	none	15 min.	30 min.	
2b	idem	sandy soil	5 hrs		
2c	distilled water	none			5 hrs

*) Concentrations are given in grams of silver per cubic centimeter of water.

Table XXXIX (continued)

exp. no	solution tested	lysimeter treatment	movements ceased after	rigor mortis	duration of exp.
3a	saturated silver solution (4 months old)	none	5½ hrs		
3b	idem	sandy soil			
3c	distilled water	none			6 hrs
4a	saturated silver solution (fresh)	none		45 min	
4b	idem	sandy soil (washed)			
4c	distilled water	none			2½ hrs
5a	saturated silver solution (fresh)	none		45 min	
5b	idem	sandy soil (burned)			
5c	distilled water	none			1½ hrs
6a	Ag-prot. 1 x 10^{-4}	none		30 min	
6b	idem	sandy soil (burned and washed)			
6c	distilled water	none			24 hrs
7a	saturated silver solution (fresh)	none		45 min	
7b	idem	glass sand			
7c	distilled water	none			19 hrs
8a	saturated silver solution (fresh)	none		40 min	
8b	idem	copper wire gauze			
8c	distilled water	none			4 hrs
9a	fresh saturated silver sol. with one drop 0.1 N copper nitrate	none		45 min	
9b	one drop of 0.1 N $Cu(NO_3)_2$ in 5 cc.	none		60 min	
9c	distilled water	none			1 hr

Table XXXIX (continued)

exp. no	solution tested	lysimeter treatment	movements ceased after	rigor mortis	duration of exp.
10a	saturated silver chloride solution 1.25×10^{-5}	none		30 min	
10b	idem	none		45 min	
10c	idem	river sand (washed)	3 hrs	5½ hrs	
10d	idem	idem	3½ hrs	6 hrs	
10e	distilled water	none			6 hrs
11a	saturated silver chloride solution 1.25×10^{-5}	none	15 min	30 min	
11b	idem	sandy soil	2 hrs	4½ hrs	
11c	distilled water	none			30 hrs
12a	saturated silver chloride solution 1.25×10^{-5}	none	20 min	45 min	
12b	idem	red alluvial soil	2 hrs	3½ hrs	
12c	distilled water	none			4 hrs
13a	Ag-prot. 1×10^{-5} plus equival. wght sodium chloride	none	6½ hrs		
13b	idem	red alluvial soil	7 hrs		
13c	distilled water	none			18 hrs
14a	silver chloride precipitated from Ag-prot. 1×10^{-5}	none	25 min	50 min	
14b	idem	sandy soil			
14c	distilled water	none			21 hrs
15a	saturated solutions of silver hydroxide and silver chloride	none	20 min	1½ hrs.	
15b	idem	black turf soil	4½ hrs	12 hrs	
15c	distilled water	none			12 hrs
16a	zinc chloride 1×10^{-5}	none			

Table XXIX (continued)

Exp. no	solution tested	lysimeter treatment	movements ceased after	rigor mortis	duration of exp.
16b	equal parts of zinc chloride 1 x 10^{-5} and sat. silver sol.	none	25 min	1¾ hrs	
16c	saturated silver solution	none	25 min	50 min	
16d	distilled water	none			12 hrs
17a	zinc chloride 1 x 10^{-5}	black turf soil			
17b	distilled water				11 hrs
18a	saturated solutions of silver hydroxide and silver chloride	none	20 min	60 min	
18b	idem	black turf saturated with $ZnCl_2$ and dried	50% dead after 2¼ hrs		
18c	distilled water	none			2¾ hrs
19a	dry mixture of 1 gm Ag-prot. and 1 gm potassium chlorate	mixed undissolved with sandy soil then tap water percolated			
19b	distilled water	none			20 hrs
20a	1 gm Ag-prot. and 1 gm potassium chlorate dissolved in 1400 cc tap water	none	1½ hrs		
20b	idem	sandy soil			
20c	distilled water	none			18 hrs
21a	saturated solutions of silver hydroxide and silver chloride plus $ZnCl_2$ 1 x 10^{-5}	none	3 hrs	none	
21b	idem	red alluvial soil	10 hrs		
21c	distilled water	none			10 hrs
22a	saturated solutions of silver chloride and lead nitrate	none	20 min	3 hrs	
22b	idem	red alluvial soil	2 hrs	none	

Table XXXIX (continued)

exp. no	solution tested	lysimeter treatment	movements ceased after	rigor mortis	duration of exp.
22c	distilled water	none			24 hrs
23a	Ag-prot. 1 x 10^{-6} and saturated sol. of lead nitrate	none	15 min	60 min	
23b	idem	red alluvial soil			
23c	distilled water	none			10 hrs
24a	saturated solution of silver carbonate 2.48 x 10^{-4}	none		30 min	
24b	idem	red alluvial soil			
24c	distilled water	none			17 hrs
25a	0.0025 % silver precipitate in tap water	none			
25b	idem plus 0.5 % sodium chloride	red alluvial soil. 1st drops			
25c	idem	idem last drops			
25d	tap water	idem			19 hrs
26a	silver nitrate in tap water 25 x 10^{-4}	none		8 hrs	
26b	idem	1 lb red alluvial soil. 1st drops			
26c	idem	idem last drops			
26d	distilled water	none			24 hrs
27a	Ag-prot. 1 x 10^{-5}	none		60 min	
27b	idem	1 lb red alluvial soil. 1st drops			
27c	idem	idem last drops			
27d	distilled water	idem			9 hrs
28a	Ag-prot. 1 x 10^{-5} plus 1 N sodium cyanide	none	2 min		
28b	idem	none	2 min		

Table XXXIX (continued)

exp. no	solution tested	lysimeter treatment	movements ceased after	rigor mortis	duration of exp.
28c	distilled water	none			9 hrs
29a	sodium cyanide 0.0088 %	none	instantly stiffened	recovered completely	4 hrs
30a	Ag-prot. 1 x 10^{-6} plus equivalent wgt sodium cyanide	none			
30b	idem	2 lbs red turf soil	3 hrs	15 hrs	
30c	distilled water	none			16 hrs
31a	Ag-prot. 1 x 10^{-5} plus eq. wght NaCN in tap water	none	16 hrs		
31b	idem	2 lbs red turf soil	7½ hrs		
31c	distilled water	none			16 hrs

Experiment 31 was four times repeated with the same result.

32a	Ag.prot. 1 x 10^{-6} plus eq. wght NaCN in tap water	none			
32b	idem	2 lbs red turf soil		80 after 24 hrs	
33a	Ag-prot. 1 x 10^{-5} plus eq. wght NaCN and excess NaCl	none		11 hrs	
33b	idem	2 lbs red turf soil	1½ hrs		
33c	distilled water	none			20 hrs
34a	Ag-prot. 1 x 10^{-5} plus eq. wght NaCn and 0.1 % $ZnCl_2$	none	8 hrs	17 hrs	
34b	idem	2 lbs red turf soil	4 hrs	10 hrs	
34c	zinc chloride 0.1 %	idem			
34d	distilled water	none			39 hrs

Experiments 33 and 34 were each four times repeated simultaneously with consistent results as above. Each set of experiments with the same number was always carried out simultaneously and observed for a length of time as indicated in the last column.

DISCUSSION OF RESULTS The lysimeter experiments showed distinctly that adsorption takes place in the soil, so much even, that solutions with silver ions only become completely impotent *) (experiments 1b, 4b, 27b, c) unless in strong concentrations (exp. 2b). Even washed soil (exp. 4b), burned soil (to destroy organic matter: exp. 5b) or soil burned and washed (i.e. freed of colloidal and organic particles: exp. 6b) had the same effect. But not only soil, glass sand (exp. 7b) and copper wire gauze (exp. 8b) as well, adsorbed the ions sufficiently as to render them inactive. The copper ion as such was not responsible for the inactivation as shown by experiment 9a.

In the presence of chlorine or cyanide ions, however, the oligodynamic action was patent (experiments 10c, d, 11b, 12b, 13b, 15b, 18b, 21b, 22b, 30b, 31b, 32b, 33b and 34b), although a significant decrement in effectiveness was observed (compare the last series with a of the corresponding numbers: the solutions tested before percolation through the lysimeter). **)

This rather unexpected result needs further attention. If (as in the absence of chlorine or cyanide) all the silver is taken out of the solution by adsorption to soil particles and colloids, the fact that presence of chlorine or cyanide ions brings about an oligodynamic effect would suggest that at least a certain amount of silver remains in solution as a result of the interaction of cations and anions. One could explain this by assuming that the affinity of silver to the anions in question is greater than its affinity to other particles. In this

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*) This adsorption explains negative results with silver-proteinate as obtained by Jacks (1944) in pot experiments, by myself (1941, unpublished) in tomato seedbed trials, and others (personal communications).

**) That actually the free chlorine ion has to be present, was shown by experiments 19a and 20b, in which the chlorate ion was of no influence.

case, however, the oligodynamic potency of a solution containing silver and chlorine ions should be equal to the toxicity of a silver chloride solution, which is definitely not what I could observe.

According to the law of mass action (Guldberg & Waage) the solubility product of an electrolyte, or the product of the concentrations of cations and anions, is a constant. This means that any excess of anions (and we may assume a large number of anions to be present in the soil) would automatically reduce the concentration of the free cations of a badly soluble metal, in casu the silver, which, therefore, will cause a considerable decrease in the efficiency of the oligodynamicon. This explains the decrement in action of a percolated solution when compared with its original strength. But the law of mass action in its original form can not explain why an increased concentration of chlorine (or cyanide) ions enhances the oligodynamic effect. Only the more recent extensions of the law of mass action (detailed discussion of which can not be given here) give full account of the behaviour of the ions when namely the activity co-efficient of the ions *) is taken into consideration.

The observed phenomenon is in physical chemistry referred to as the s.c. "salt effect" (see Vogel, 1943) which throws new light on the oligodynamic action of metal ions, in that - on special conditions of electrolytical equilibrium - certain ions may take the role of what could be called a "synergon".

A synergon can now be defined as an ion that augments the solubility of an oligodynamicon, thus keeping it potent in an environment where its activity is impeded.

Experiments 23b, 24b and 26b, c showed that this protective power is not possessed by the nitrate and carbonate anions, and Just & Sapolin (1936a) found that the presence of iodine ions

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*) A concept introduced by G.N. Lewis (1901, 1907).

considerably reduces the solubility of silver iodide which is already very low (solubility product 1.5×10^{-16} grammolecules per liter at 25° C). In fact, the halogens Cl and CN seem to be outstanding as synergon, which is perhaps explained by their comparatively high solubility.

In the compound "Protargol" the silver seems to be "shielded" to a certain extent, as addition of sodium chloride does not immediately result in the precipitation of silver chloride, although the reaction does take place after a few minutes' standing. The efficiency of the silver in "Protargol" is also decreased when its solutions are compared with other compounds containing equivalent amounts of silver (cf. experiments 27a and 10a, 11a, 12a).

"Protargol", which is normally of a dark brown colour in concentrated solutions, reacts with sodium or potassium cyanide in that the solution becomes nearly completely colourless. Most likely a complex silver ion is formed, as no precipitate ensues. Consequently the oligodynamic action of solutions of "Protargol" in combination with NaCN is somewhat surprising, since we have seen from experiments 30a and 32a that the complex silver ion is inactive (unless sufficiently dissociated after a long time: exp. 31a; see also page 91). Evidently, then, is this complex ion broken up in the soil, so that free silver ions are liberated. Otherwise the results of experiments 30b, 31b and 32b can not be explained.

The "shielded" position of the silver in the complex ion is an advantageous feature, as it allows the simultaneous administration in the soil of oligodynamicon and synergon without risking localized precipitations of slowly soluble compounds. This principle has been used in later investigations.

Neither the duration of percolation through the lysimeter, nor the amount of soil taken seemed to be of any influence on the "soil effect" (experiments 25 - 27) and different soils appeared to behave alike with respect to adsorption.

SILVER-ZINC CYANIDE, A PROMISING COMPOUND Above we have seen that the complex silver ions have certain advantageous properties that would make them better nematocides than other silver compounds. The idea, therefore, to look for a substitute of the oligodynamically inactive alkali atoms in the silver-alkali cyanides, was obvious. Naturally zinc suggested itself here, as this metal too exerted detrimental influence on the eelworm larvae *). I thus tried the synthesis of a complex ion of silver and zinc and succeeded in precipitating a silver-zinc cyanide, $Ag_2Zn(CN)_4$, by mixing solutions of zinc nitrate and silver-sodium cyanide according to the reaction equations:

$$2AgNO_3 + 2NaCN \longrightarrow 2AgCN \downarrow + 2NaNO_3$$

$$2AgCN + 2NaCN \longrightarrow 2AgNa(CN)_2 \text{ (soluble)}$$

$$2AgNa(CN)_2 + Zn(NO_3)_2 \longrightarrow Ag_2Zn(CN)_4 \downarrow$$

Silver-zinc cyanide, a greyish powder, is highly insoluble, but can be dissolved in sodium cyanide **). It does not react with sodium chloride and is, by itself, oligodynamically inactive. Compared with other silver compounds it is much less photo-sensitive. All this shows that the silver atom is well shielded off.

With some experimentation the Department of Chemical Services, Pretoria, ultimately evolved four different ways of preparing silver-zinc cyanide and mass production of the compound actually appeared to be a rather simple procedure.

FIELD AND SEEDBED TRIALS A series of experiments was set out to investigate the practical applicability of different silver compounds. The following chemicals were tested: metallic silver (silver dust), silver proteinate

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*) Another property of zinc is that it tends to displace silver ions, which - it was hoped - might prevent adsorption of silver in the soil.

**) In fact complex cyanides of a higher order are formed in this way.

("Protargol", a commercial product containing about 8 % silver), silver nucleinate (with ± 16 % Ag), silver-zinc cyanide and silver chloride. Sodium chloride was used as a synergon and tried out in different concentrations, while sodium cyanide was applied either as synergon or as a solvent of the insoluble compounds.

As a statistical lay-out I adopted the randomized block system. In this way the host plants, examined in the laboratory some time after treatment of the soil (2 - 3 months), can be taken as random samples of the eelworm population present in the soil at the moment when invasion of the roots was started by the parasites. The controls then give an estimate of the population density prior to treatment.

Provisions were made to have N (the number of plants exposed to infestation) large, so that formula (22) could be applied as an estimate of eelworm population.

Since the N's varied from plot to plot (and thus also for the different treatments) it was deemed essential here to take as a criterion the average, Σ/N of $-\ln(1 - n/N)$, of all the indicator plants for each treatment, divided by the corresponding figure for the control *). By this operation the index figures come to represent a percentage of the original population that survived treatment, for which reason I designated them as "survival indices".

As a unit of treatment dosage was chosen the amount of silver present in 5 grams of silver proteinate (which corresponds to 0.4 gm of silver) administered per square yard of eelworm infested soil. This one unit is equal to: 5 gm "Protargol", 2.5 gm silver nucleinate, 0.7 gm silver-zinc cyanide or 0.5 gm silver chloride per square yard. Unit of synergon was 5 gm sodium chloride, and likewise sodium cyanide was reckoned in units of 5 gm per square yard.

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*) When N is constant for all treatments it falls out automatically.

The results of these experiments are shown below.

Table XL

exp. no.	treatment and doses in units per square yard	n = plants infest.	N = total plants	$\frac{n}{N}$	$-\ln(1-\frac{n}{N})$	survival index
I_1	silver nucleinate 0.25 in 18.6 gm bloodmeal a)	21	32	0.656	1.0671	83.6
I_2	control	35	50	0.700	1.2040	100.0
II_1	silver dust 0.35 in 18.6 gm bl.meal	23	40	0.575	0.3557	82.0
II_2	control	166	256	0.648	1.0441	100.0
III_1	silver proteinate 0.25 in 18.6 gm bloodmeal	9	123	0.073	0.0758	88.6
III_2	control	44	538	0.082	0.0856	100.0
IV_1	"Protargol" 0.5 NaCN 0.5 NaCl 0.5	867 242 199 245 240 1793	1185 1591 665 588 1816 5845	0.307	0.3667	73.5
IV_2	"Protargol" 1.0 NaCN 1.0	40 123 120 88 27 398	639 1136 735 455 79 3044	0.131	0.1404	28.1
IV_3	"Protargol" 1.0 NaCN 1.0 NaCl 1.0	15 158 108 114 32 427	1098 437 838 852 495 3720	0.115	0.1228	24.6
IV_4	"Protargol" 0.5 NaCN 0.5	347 507 420 271 425 1970	577 1172 1088 519 1792 5148	0.383	0.4829	96.7
IV_5	control no 1 NaCN 1.0	702 79 699 358 251 2089	996 1400 2055 493 356 5300	0.394	0.5009	
IV_6	control no 2 no treatment	92 608 228 329 297 1554	1017 923 1112 537 375 3964	0.392	0.4976	

a) Bloodmeal contains 0.87 % chlorine.

Table XL (continued)

exp. no.	treatment and dosage in units per square yard	n = plants infest.	N = total plants	$\frac{n}{N}$	$-\ln(1-\frac{n}{N})$	survival index
IV_5-IV_6	control for IV_{1-4}	3643	9264	0.393	0.4992	100.0
V_1	"Protargol" 2.0 NaCN 2.0 NaCl 4.0	83 255 264 606	287 809 429 1545	0.388	0.4910	9.6
V_2	"Protargol" 2.0 NaCN 2.0 NaCl 6.0	190 584 554 1328	409 661 1213 2283	0.582	0.8723	17.1
V_3	"Protargol" 3.0 NaCN 3.0 NaCl 3.0	429 94 316 839	643 523 1677 2843	0.295	0.3496	6.8
V_4	"Protargol" 3.0 NaCN 3.0 NaCl 6.0	415 777 192 1384	1681 1370 863 3914	0.354	0.4360	8.5
V_5	"Protargol" 3.0 NaCN 3.0 NaCl 9.0	434 212 232 878	735 427 449 1611	0.545	0.7897	15.4
V_6	$Ag_2Zn(CN)_4$ suspension 1.0 NaCl 2.0	297 227 460 984	309 233 476 1018	0.967	3.4112	66.7
V_7	$Ag_2Zn(CN)_4$ suspension 2.0	344 402 767 1513	373 414 817 1604	0.943	2.8647	56.0
V_8	$Ag_2Zn(CN)_4$ suspension 2.0 NaCl 2.0	206 256 424 886	610 770 570 1950	0.454	0.6051	11.8
V_9	$Ag_2Zn(CN)_4$ suspension 2.0 NaCl 4.0	483 872 166 1521	648 1502 477 2627	0.579	0.8651	16.9
V_{10}	$Ag_2Zn(CN)_4$ suspension 2.0 NaCl 6.0	344 326 250 920	659 461 323 1443	0.638	1.0161	19.9
V_{11}	$Ag_2Zn(CN)_4$ suspension 3.0	168 670 246 1084	233 771 570 1574	0.689	1.1660	22.8

Table XL (continued)

exp. no.	treatment and dosage in units per square yard		n = plants infest.	N = total plants	$\frac{n}{N}$	$-\ln(1-\frac{n}{N})$	survival index
V_{12}	$Ag_2Zn(CN)_4$ suspension	3.0	355	571			
	NaCl	3.0	481	1257			
			133	599			
			969	2427	0.399	0.5092	10.0
V_{13}	$Ag_2Zn(CN)_4$ suspension	3.0	358	1177			
	NaCl	6.0	770	1148			
			695	883			
			1823	3208	0.568	0.8393	16.4
V_{14}	$Ag_2Zn(CN)_4$ suspension	3.0	828	1047			
	NaCl	9.0	1384	2321			
			895	2123			
			3107	5491	0.566	0.8347	16.3
V_{15}	control for V_{1-14}		185	186			
			188	190			
			154	154			
			3	550	0.994	5.1160	100.0
VI_1	AgCl	1.0					
	NaCN	1.0	9	43			
			0	5			
			16	28			
			5	16			
			1	9			
			0	16			
			31	117	0.265	0.3079	83.0
VI_2	AgCl	4.0					
	NaCN	4.0	1	19			
			8	28			
			2	20			
			12	88			
			5	11			
			0	37			
			28	203	0.138	0.1485	40.0
VI_3	"Protaryol"	0.5					
	NaCN	0.5	33	69			
			5	13			
			6	17			
			16	64			
			2	4			
			1	75			
			63	242	0.260	0.3011	81.1
VI_4	"Protargol"	4.0					
	NaCN	4.0	13	44			
			0	1			
			1	17			
			0	35			
			0	46			
			3	40			
			17	183	0.093	0.0976	26.3
VI_5	$Ag_2Zn(CN)_4$	4.0					
	NaCN	4.0	3	48			
			9	37			
			3	38			
			0	26			
			1	47			
			0	58			
			16	254	0.063	0.0651	17.5

Table XL (continued)

exp. no.	treatment and dosage in units per square yard	n = plants infest.	N = total plants	$\frac{n}{N}$	$-\ln(1-\frac{n}{N})$	survival index
VI_6	$Ag_2Zn(CN)_4$ dry powder 4.0	0	2			
		4	6			
		0	1			
		2	10			
		3	8			
		1	13			
		10	42	0.238	0.2718	73.2
VI_7	$Ag_2Zn(CN)_4$ 0.5 NaCN 0.5 NaCl 0.25	39	73			
		0	6			
		4	28			
		8	68			
		13	68			
		4	286			
		73	529	0.138	0.1485	40.0
VI_8	$Ag_2Zn(CN)_4$ 2.0 NaCN 2.0 NaCl 1.0	3	32			
		6	34			
		28	67			
		1	53			
		3	354			
		0	14			
		41	554	0.074	0.0769	20.7
VI_9	$Ag_2Zn(CN)_4$ 4.0 NaCN 4.0 NaCl 2.0	3	40			
		3	11			
		0	30			
		0	49			
		1	100			
		1	36			
		8	286	0.030	0.0305	8.2
VI_{10}	$Ag_2Zn(CN)_4$ 1.0 NaCN 1.0 NaCl 0.25	6	7			
		3	25			
		1	9			
		3	42			
		0	21			
		1	27			
		14	131	0.107	0.1132	30.5
VI_{11}	$Ag_2Zn(CN)_4$ 2.0 NaCN 2.0 NaCl 0.5	7	96			
		5	41			
		3	5			
		0	56			
		0	97			
		0	328			
		15	623	0.024	0.0243	6.5
VI_{12}	control for VI_{1-11}	71	130			
		22	41			
		7	9			
		10	66			
		39	284			
		8	46			
		160	516	0.310	0.3711	100.0

Table XL (continued)

exp. no.	treatment and dosage in units per square yard	n = plants infest.	N = total plants	$\frac{n}{N}$	$-\ln(1-\frac{n}{N})$	survival index
VII_1 *)	AgCl 5.09 NaCN 5.09	11	54			
		10	47			
		7	35			
		6	41			
		9	41			
		9	32			
		8	37			
		7	48			
		63	355	0.177	0.1948	5.7
VII_2 *)	$Ag_2Zn(CN)_4$ 4.92 NaCN 4.92 NaCl 2.63	13	57			
		1	42			
		9	44			
		2	55			
		19	63			
		3	56			
		3	53			
		1	53			
		51	423	0.121	0.1290	6.4
VII_3 *)	control for VII_{1-2}	51	56			
		48	61			
		47	52			
		45	48			
		42	60			
		55	60			
		44	56			
		58	58			
		390	451	0.865	2.0025	100.0
$VIII_1$ *)	AgCl 5.09 NaCN 5.09	1	50			
		0	50			
		1	50			
		0	50			
		6	50			
		2	50			
		0	50			
		0	50			
		10	400	0.025	0.0253	2.6
$VIII_2$ *)	$Ag_2Zn(CN)_4$ 4.92 NaCN 4.92 NaCl 2.63	9	50			
		2	50			
		0	50			
		1	50			
		2	50			
		0	50			
		5	50			
		1	50			
		20	400	0.050	0.0513	5.3

*) Figures by courtesy of Mr A.J. Smith (Rustenburg) and Dr E.J. van der Linde (Pretoria) from experiments carried out at Rustenburg (VIII) and Brits (VII) according to specifications by the author.

Table XL (continued)

exp. no.	treatment and dosage in units per square yard	n = plants infest.	N = total plants	$\frac{n}{N}$	$-\ln(1-\frac{n}{N})$	survival index
$VIII_3$ *)	control for $VIII_{1-2}$	40	50			
		12	50			
		30	50			
		16	50			
		22	50			
		38	50			
		39	50			
		50	50			
		247	400	0.618	0.9623	100.0

Experiments I - III were carried out by means of spot treatment in fields planted to Turkish tobacco 3 x 3 feet. The dosage was administered in powder form, mixed with the fertilizer (bloodmeal). The latter was given at the rate of 200 lbs per acre (± 18.6 gm per plant).

Plant heights (both treated and controls) were measured in experiments II and III. For treatment II_1 (silver dust), the average length was 40 inches (ranging from 4 to 64") and for the controls (II_2) 37 inches (ranging from 5 to 60"). For treatment III_1 ("Protargol") the average length was 24 inches (3 to 52") and for its control (III_2) 13 inches (1 to 40"). In either case the differences were not significant (as the variances were very large) but showed at least that the silver had no adverse effect on the growth of the tobacco plants. If not directly stimulating growth, silver showed to be effective with respect to eelworm infestation and the slight reduction of root damage could possibly account for the differences in length.

All the other experiments (IV - VIII) were tobacco seedbed trials in randomized blocks. In experiment IV the plots were 3 x 15 ft (5 square yards) while treatment was given in aqueous solution. As treatment IV_5 (1 unit of NaCN) showed practically speaking no difference with the controls (IV_6), the average of IV_5 and IV_6 was taken as a base for the computation of the sur-

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*) See foot note preceding page.

vival indices of treatments IV_1 to IV_4.

The plots of experiment V were 3 x 9 ft (3 square yards). "Protargol" was administered in aqueous solution and silver-zinc cyanide in watery suspension (prepared by shaking up the powder with a little absolute alcohol; the mixture was then poured out into 4 gallons of water while stirred; finally NaCl was added).

For experiment VI the plots were taken one square yard in size, but more replications occurred. Dosage was given by means of aqueous solution of the chemicals (except for treatment VI_6 in which the dry powder of silver-zinc cyanide was worked up with the top 3" layer of soil).

In experiments VII and VIII the plots were two square yards and treatment was given in aqueous solution.

With treatments IV - VI the total number of plants in the plots were examined, while for experiments VII and VIII samples of size N were taken from the replications. As in all cases N was large and taken from representative areas in the experimental field, the various ways of sampling did not seem to have been of influence on the results. From experience, however, and on theoretical grounds, I would recommend to have as large a number of replications as the experimental lay-out will permit. The ideal would be a large number of plots, distributed at random over the experimental area, and in general one could say: the smaller the plots, the larger should be the number of replications.

DISCUSSION OF RESULTS When plotting the survival indices of a series of experiments, as e.g. III_1, IV_1, IV_3, V_1 ("Protargol") or VI_7, VI_8, VI_9, VII_2, $VIII_2$ (silver-zinc cyanide), against units of silver administered, long drawn-out, sigmoid curves result, which become symmetrical on logarithmic transformation of the doses. This trend is typical of many toxicological data, and indicates that, in the cases

FIG. 8

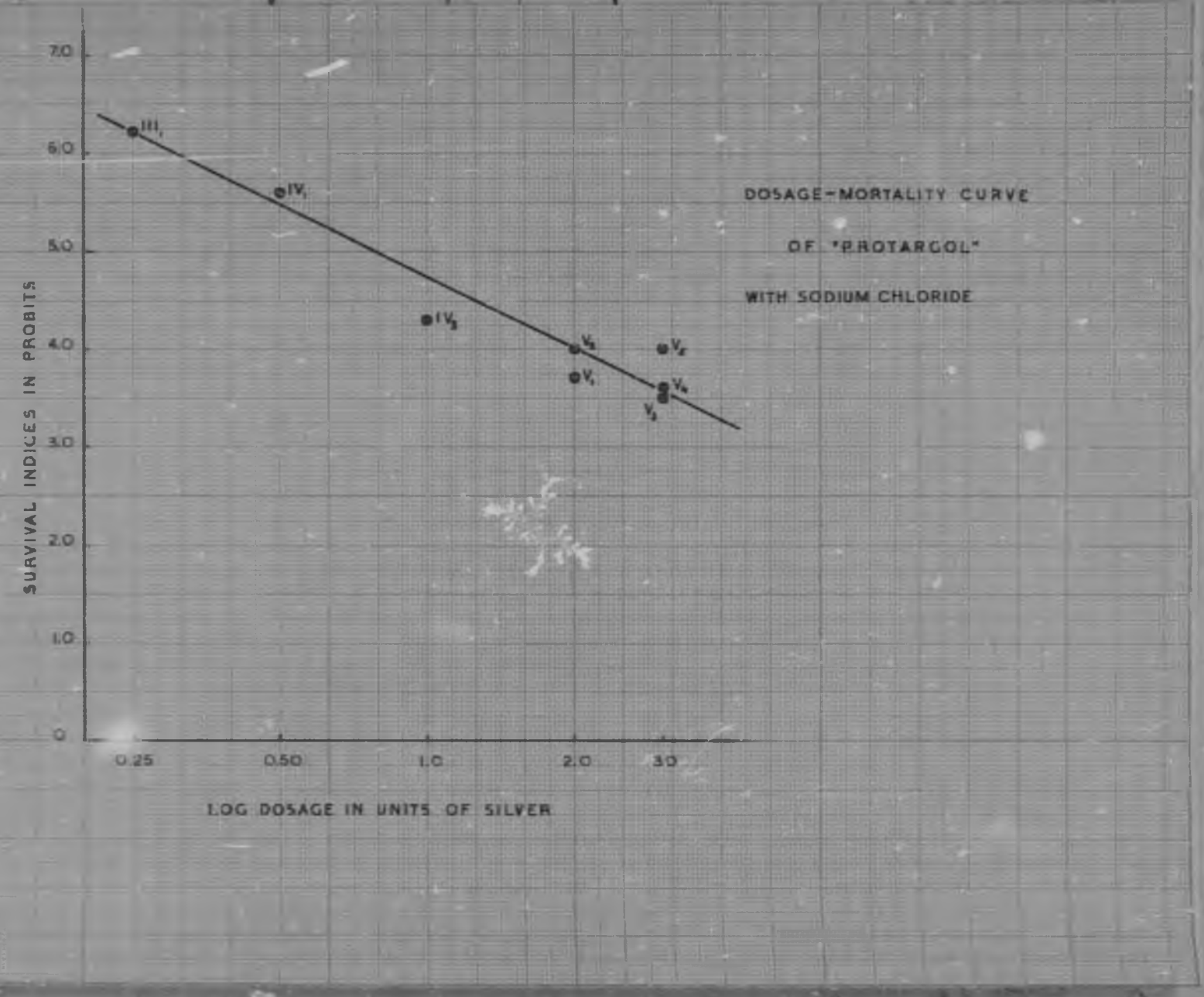
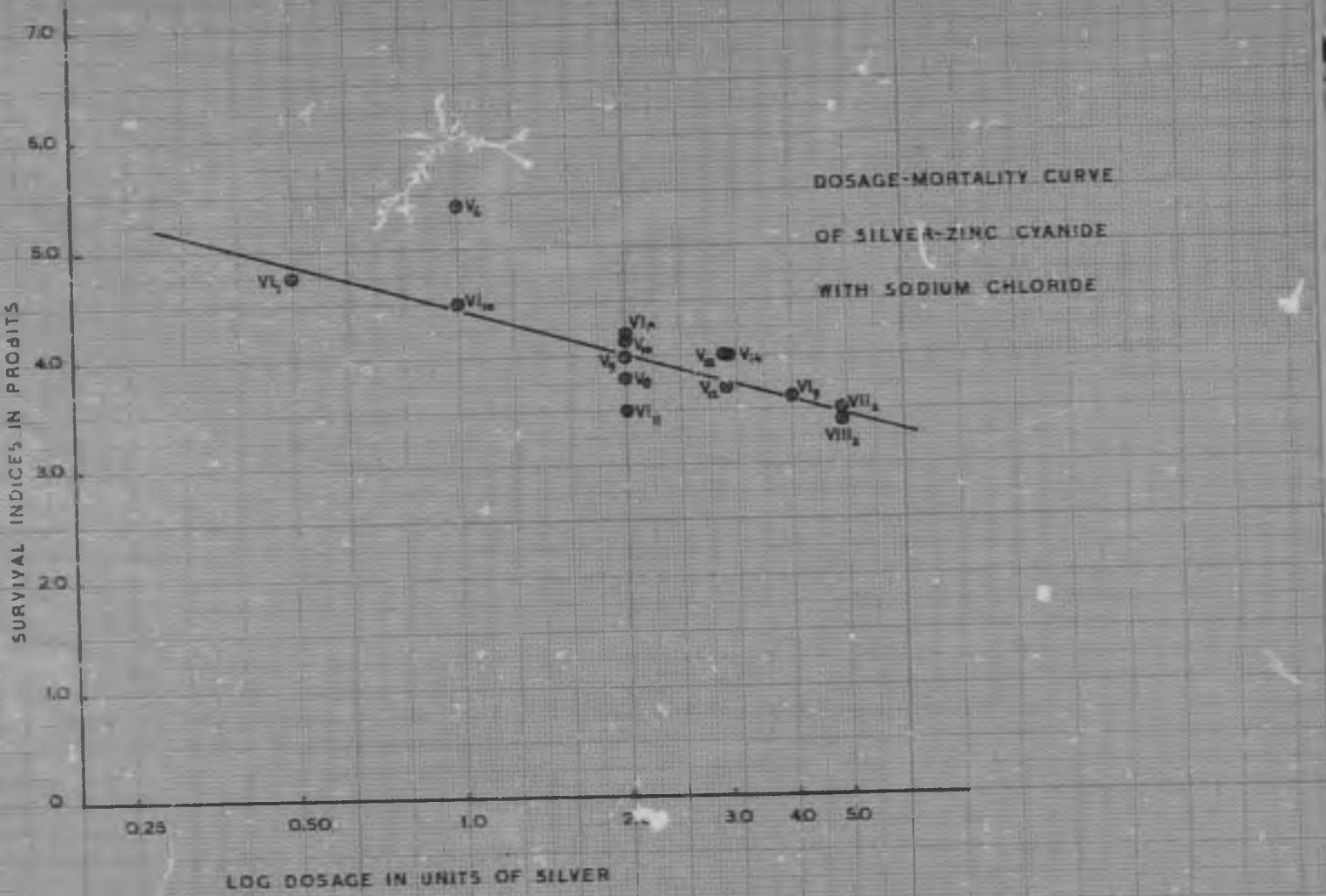

DOSAGE-MORTALITY CURVE
OF "PROTARGOL"
WITH SODIUM CHLORIDE
SURVIVAL INDICES IN PROBITS
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
III
IV
IV3
V2
V5
V1
V4
V3
0.25
0.50
1.0
2.0
3.0
LOG DOSAGE IN UNITS OF SILVER

FIG. 9


DOSAGE-MORTALITY CURVE
OF SILVER-ZINC CYANIDE
WITH SODIUM CHLORIDE
SURVIVAL IN PROBITS
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0.25
0.50
1.0
3.0
4.0
5.0
LOG DOSAGE IN UNITS OF SILVER

● SILVER DUST

○ SILVER NUCLEINATE

● WITHOUT SODIUM CHLORIDE

● SILVER CHLORIDE

● SILVER PROTEINATE

● SILVER-ZINC CYANIDE

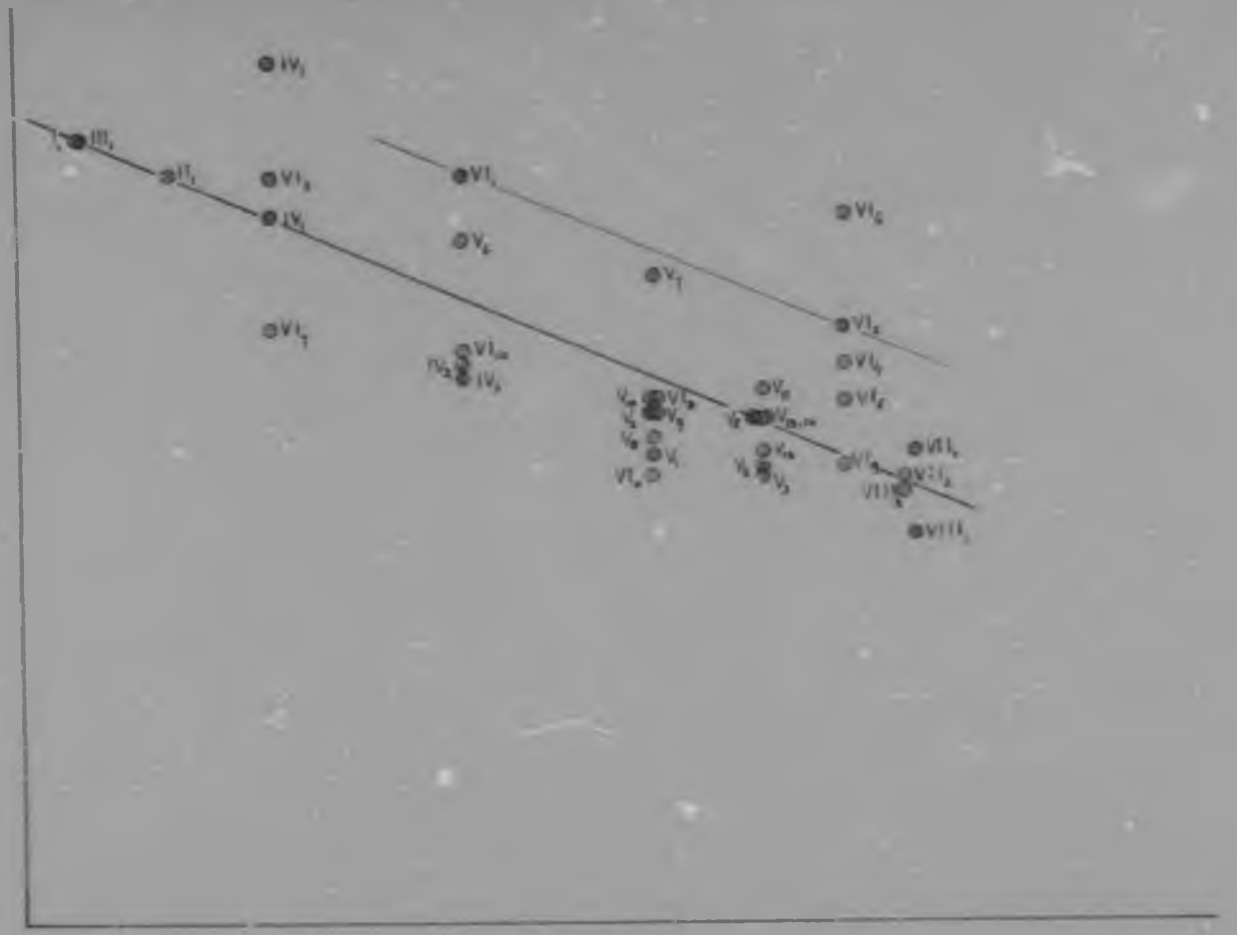


FIG. 10

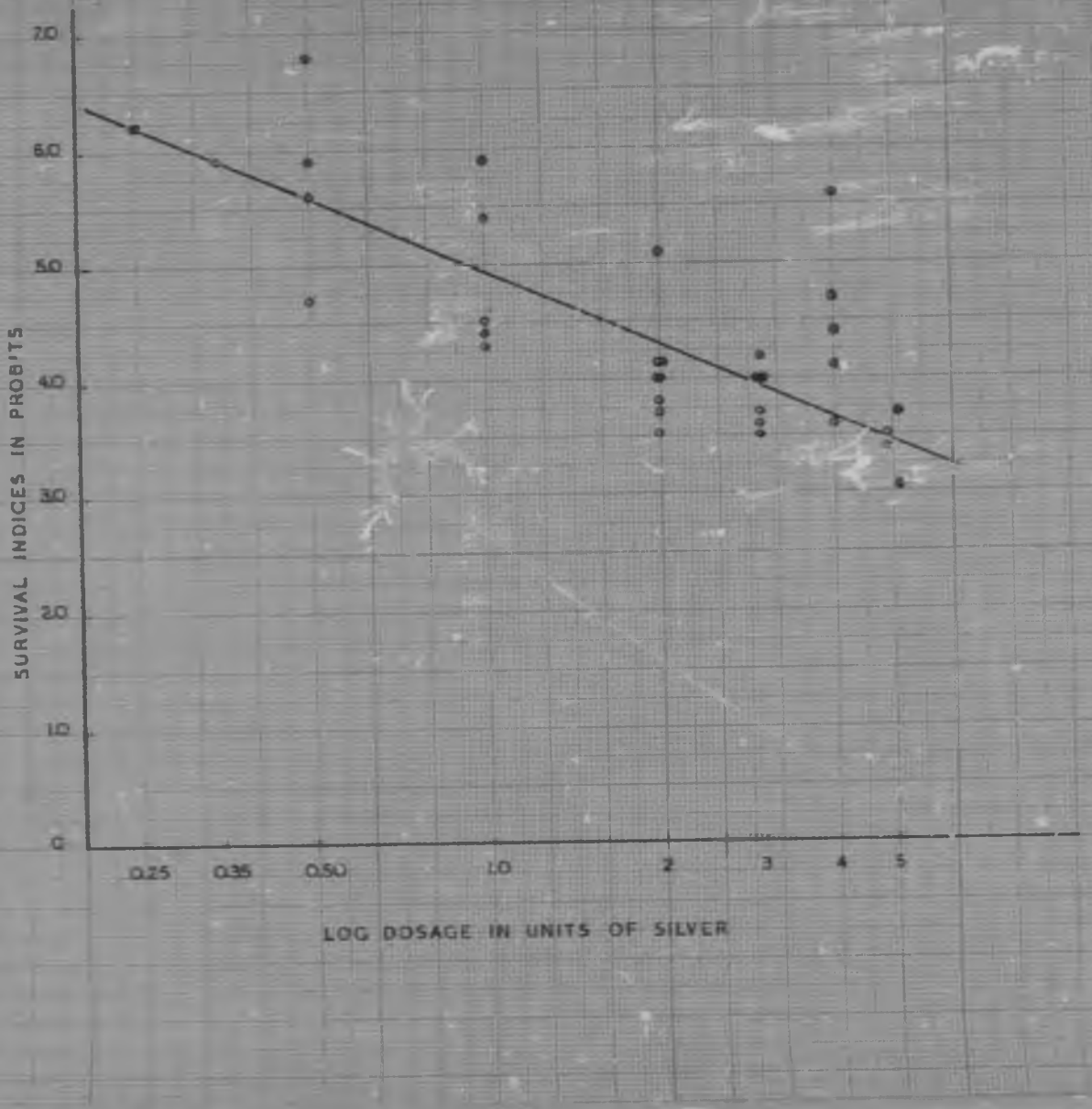
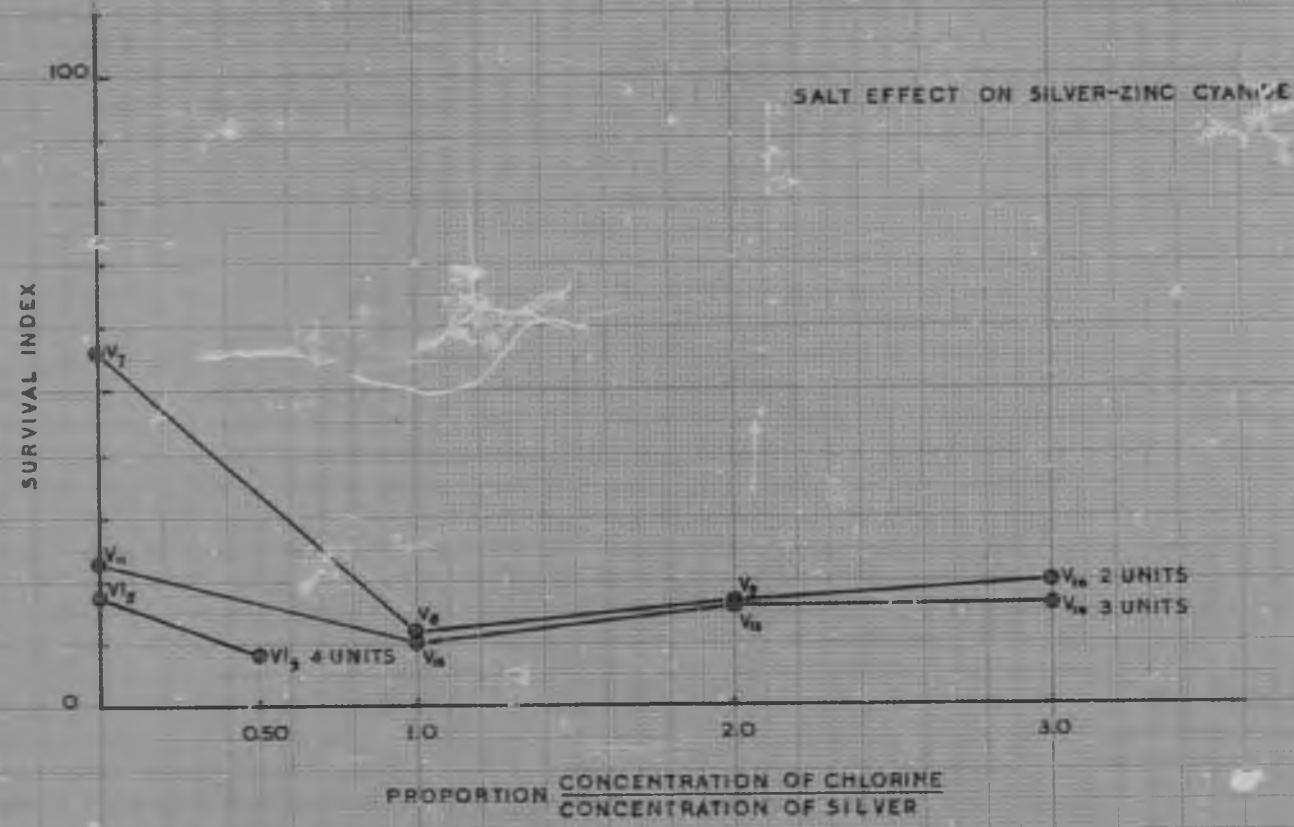

70
60
50
40
30
20
10
0
SURVIVAL INDICES IN PROBITS
0.25
0.35
0.50
1.0
2
3
4
5
LOG DOSAGE IN UNITS OF SILVER

FIG. II


SALT EFFECT ON SILVER-ZINC CYANIDE
100
SURVIVAL INDEX
0
0.50
1.0
2.0
3.0
PROPORTION CONCENTRATION OF CHLORINE / CONCENTRATION OF SILVER
4 UNITS
2 UNITS
3 UNITS

in which it is observed, the efficiency of the dosage is concordant with the law of Weber-Fechner, which (when expressed in general terms) postulates that in multicellular organisms the response to external influences is proportional to the logarithm of their strength. Though from theoretical points of view many plausible objections have been raised against this law, one can not do away with the fact that time and again it appears to offer a most useful description of the experience, so that it has maintained a certain significance in practical physiology, toxicology and applied biology. In our case applying the law would be focussed on the population of eelworms as a whole rather than its constituting individuals.

The sigmoid shape of the log-dosage mortality curves betrays that the "percentage kill' is normally distributed about the mean, which is the log-dose at 50 % kill. This enables us to rectify the dosage-mortality curve to a straight line (Gaddum, 1933; Lode, 1936) by taking probability units (or "probits") as ordinates instead of percentage survival (Bliss, 1934, 1935 a, b). Tables of "probits" are found in Fisher & Yates (1938). Figures 8 and 9 show regression lines obtained in this way for "Protargol" and silver-zinc cyanide respectively. Figure 10 represents the information from table XL in log-dosage probit transformation. The drawn out line shows the estimated general trend of regression and should not be regarded as of any more significance than this. It enables us, however, to gauge the - rather scattered - results listed in table XL.

First of all the "salt effect" is well exhibited in fig. 10. It will be seen that 7 of the 8 treatments without sodium chloride yielded survivals above the line of general trend, which stresses once more the important function of chlorine as a synergon. But for best results sodium chloride can not be given in any proportion with the silver compounds. There is a definite progress in the efficiency of the silver ions when the proportion $[Ag^+]/[Cl^-]$ increases. This is shown by fig. 11 and further by

comparison of the treatments in the following series: V_1, V_2; V_3, V_4, V_5; IV_1, IV_4; IV_2, IV_3.

Apparently we have, for optimum efficiency, a critical proportion of the concentrations of the two kinds of ions, which also theoretically was to be expected. I have not yet had the opportunity to determine the exact value of this critical proportion.

The results obtained with silver chloride were somewhat disappointing. The chemical was dissolved in sodium cyanide, which brings silver-sodium cyanide and NaCl in solution, so that an effect similar to the other compounds in conjunction with NaCl could be expected. The variance in efficiency appeared to be large and more data are required before this can be explained.

Comparison of figures 8 and 9 would suggest that "Protargol" has a slightly more drastic effect than silver-zinc cyanide, as indicated by the steeper slope in regression of the former. Silver-zinc cyanide, however, revealed an increased oligodynamic action in confirmation of the expectation when this compound was first considered (cf. page 101). This is expressed by the unit value of the mean dosage, which is very much smaller for $Ag_2Zn(CN)_4$ than for "Protargol". Consequently silver-zinc cyanide, when repeatedly administered in very small quantities ($\pm$ 0.25 units) - dissolved in irrigation water e.g. - should be most advantageous.

Considering the difference in survival indices of treatments VI_5 (4 units dissolved $Ag_2Zn(CN)_4$) and VI_6 (4 units undissolved), the necessity of applying the chemical in solution will be evident.

The practical possibilities of soilworm control by means of oligodynamic action of silver ions is generally demonstrated by fig. 10 and more particularly by figures 8 and 9.

SUMMARY AND CONCLUSIONS Summarizing the information obtained from the experiments described in this paper leads to the following conclusions.

1) Since the distribution law for root damage

$$P(x) = \int_{x}^{\infty} cbx^{b-1}e^{-cx^{b}}dx,$$

based on the principle of random invasion of the host plants, could be fitted with satisfactory result to the observed infestation in at least 77 % of the cases investigated, the assumption a priori, that the terricolous larvae of Heterodera marioni are distributed at random in the soil, seems to be justified.

2) A random distribution of the larvae, appearing to be non-homogeneous, can only be explained by limited movements of the eelworms through the soil.

3) According to the theory of probability (Thompson, 1924, 1939) the relative strength of a population of parasites (E) in random search for hosts should be a function of the number parasitized (n) and the total number of hosts (N), viz.

$$E = -N\ln(1 - n/N)$$

Estimated by means of graded root damage (x_1), the same magnitude is given by the sum total of the products of observed frequency and class weight: $S(f_1w_1) = J_1$ or $S(f_1x_1)$.

As both estimates (J_1 and E) were very highly correlated ($r = 0.98$), corroborative evidence is supplied in favour of Thompson's approximation and, consequently, in favour too of randomness in search for hosts and randomness in distribution of the terricolous larvae.

4) Having found E to be highly correlated with Godfrey's (1934 a) gall count as well ($r = 0.97$), this estimate of eelworm population strength may be used with confidence, i.e. when N is large.

5) E yields a reliable nematode index that can be quickly determined and has the very important concomitant advantage

of being independent from grading of root damage, a time-consuming and unreliable means of estimating the nematode population of the soil. Only when a large number of classes is taken and a good deal of skill has been achieved through practical experience, grading may give satisfactory results. But it will never be void of subjectivity.

6) In terms of earlier notations we may write

$$E = S(f_1 w_1) = - N \ln(1 - B)$$

when $B = n/N$ and evidently the estimated average individual infestation of plants attacked, $A = S(f_1 w_1)/n$, may now be found without grading from the formula

$$A = E/n = - N/n \ln(1 - B)$$

7) The mean infestation, formerly estimated by means of grading (Hoyer 1939) as $AB = S(f_1 w_1)/N$, is more accurately given by

$$AB = E/N = - \ln(1 - B)$$

8) The last formula above shows that the mean infestation per host plant is identical to the eelworm population mean expressed in natural units.

9) Theoretical deductions showed that this statistic is an estimate of the sole characteristic parameter of the eelworm population distribution. The statistic AB will only then represent an unbiased estimate of the true population mean when evaluated from $- \ln(1 - n/N)$ in which N should be large, and not when based on grading. Thus in general: any nematode index derived by means of graded root damage - as is general practice - should be rejected as unreliable.

10) Estimating the nematode population from n/N (= B) is also incorrect. Only very small values of B (< 0.10) are approximately equal to the mean AB (see table XL).

11) N should be a representative sample of the host plant population amongst which the eelworm population has been distributed as a result of root invasion. Such samples can be

best obtained from randomized blocks. Strictly speaking, every plant out of N examined will represent a sub-sample of the eelworm population. The standard error of its estimate, therefore, is given by the standard deviation of the mean root damage, so that we have

$$s.e._{\bar{E}} = s_{AB} = \frac{s_E}{\sqrt{N}}$$

s_E will usually be found to be rather high, which emphasizes the necessity of having N very large.

12) The proper estimation of eelworm populations in soil offered possibilities for carrying out toxicological experiments with nematocides applied to soils infested with the larvae of Heterodera marioni. Ionic silver proved to be an extremely powerful agent in vitro as well as in soil. In the latter case on condition only, that a synergon is present. Chlorine appeared to be a practical (and inexpensive!) synergon, while alkali-cyanides can be used as solvents for insoluble silver compounds.

13) Oligodynamic control of eelworms by means of silver ions proved to be possible in soil, especially when silver is applied in combination with zinc (which is also of detrimental influence to the larvae) in the form of silver-zinc cyanide. The mean dosage (securing 50 % kill) is extremely small (in the neighbourhood of 0.3 gm of $Ag_2Zn(CN)_4$ per square yard.

14) Whether silver-zinc cyanide may be regarded as an ideal nematocide, can not be judged as yet. The present paper has only given the outlines of the problem and the necessity of further research to investigate all the implications can not be sufficiently advocated.

Silver, in any case, has the advantage of being harmless to the constitution of the soil and to tobacco plants, which allows for repeated administration even during the growth of seedlings or young plants. A powerful nematocide with this property has never been described before.

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APPENDIX

For readers not familiar with statistical manipulations the formulae applied in the analysis of variance (see table I, opposite page 19) will need some explanation as I have modified Saunders' (1939) notations and carried out certain transformations.

In order to find the "sum of squared deviations from the mean" of the plot yields, Saunders takes:

$$S(x_1^2) - \bar{x}_1 S(x_1) \qquad (1)$$

In this notation $S(x_1^2)$ means the sum of the squared plot indices (the "yields"); $\bar{x}_1$ the general mean and $S(x_1)$ the grand total.

With this formula one often runs into very large figures, which are easily obviated by taking the direct formula

$$S(x_1 - \bar{x}_1)^2 \qquad (2)$$

The identity of expressions (1) and (2) will be clear when (2) is expanded as below:

$$S(x_1 - \bar{x}_1)^2 = S(x_1^2 - 2x_1\bar{x}_1 + \bar{x}_1^2) =$$
$$= S(x_1^2) - 2\bar{x}_1 S(x_1) + \bar{x}_1 S(\bar{x}_1) \qquad (3)$$

Since per definition $\bar{x}_1 = S(x_1)/m$ (when m is the number of terms to be summed, or the number of plots) and $S(\bar{x}_1) = - n\bar{x}_1$, it follows that $S(\bar{x}_1) = S(x_1)$. Substituting this into (3) we obtain:

$$S(x_1 - \bar{x}_1)^2 = S(x_1^2) - \bar{x}_1 S(2x_1 - x_1) = S(x_1^2) - \bar{x}_1 S(x_1)$$

Expression (2), apart from being easier to handle than

(1), also shows in a more straightforward way what actually is wanted, viz. the "sum of the squared deviations from the mean" of all the plot indices. These deviations will be due to:

a) controlled factors, as: 1) differences in treatment,
2) differences in position in the experimental plan (the replications); and

b) a number of unknown, and therefore uncontrolled factors, generally designated as "error".

The sum of squared deviations from the mean due to the controlled factors, then, can be analysed.

For treatments this sum of squares is given by Saunders as:

$$S S^2(x_t)/r - \bar{x}_1 S(x_1) \qquad (4)$$

in tead of which I used the formula

$$rS(\bar{x}_t - \bar{x}_1)^2 \qquad (5)$$

where $S(x_t)$ denotes the sum of the indices for each treatment, or the treatment total; $\bar{x}_t$ the treatment mean and r the number of replications.

By definition we have: $S(x_t)/r = \bar{x}_t$, whence $S^2(x_t)/r = r\bar{x}_t^2$ and, therefore,

$$S S^2(x_t)/r = rS(\bar{x}_t^2) \qquad (6)$$

For $S(x_1)$ one naturally can write $SS(x_t)$ and $S(x_t)$ may be substituted by $r\bar{x}_t$, so that we obtain

$$SS(x_t) = rS(\bar{x}_t) \qquad (7)$$

Substitution of (6) and (7) into (4) yields then

$$S S^2(x_t)/r - \bar{x}_1 S(x_1) = rS(\bar{x}_t^2) - r\bar{x}_1 S(\bar{x}_t) \qquad (8)$$

The expression to the right is of a similar type as (1), so that obviously,

$$rS(\bar{x}_t^2) - r\bar{x}_1 S(\bar{x}_t) = rS(\bar{x}_t - \bar{x}_1)^2$$

Likewise the sum of squared deviations from the mean for replications (blocks) as given by Saunders as

$$SS'(x_r)/t - \bar{x}_1 S(x_1) \qquad (9)$$

may be written as

$$tS(\bar{x}_r - \bar{x}_1)^2 \qquad (10)$$

Here t denotes the number of treatments; $S(x_r)$ the sum of the indices for each set of treatments in a replication block, or the block total, and $\bar{x}_r$ the mean of a set of treatments in a replication block.

The procedure of evaluating the sum of squared deviations from the mean due to the uncontrolled influences (error) has now become very simple in that we substract from the computed value of $S(x_1 - \bar{x}_1)^2$, (2), the values for the sums of squared deviations due to the controlled factors (treatments, (5), and replications, (10)) and obtain the required component as the residue.

The variances are found by dividing the sums of squares by the respective corresponding degrees of freedom (d.f.) whereafter the variance ratio (F) between treatment and error is used as a criterion of significance (tables of F in Fisher & Yates, 1943). P is the probability of a null hypothesis postulating no significant difference between treatments.

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CURVE FITTING by means of Sichel's method of "frequency moments".

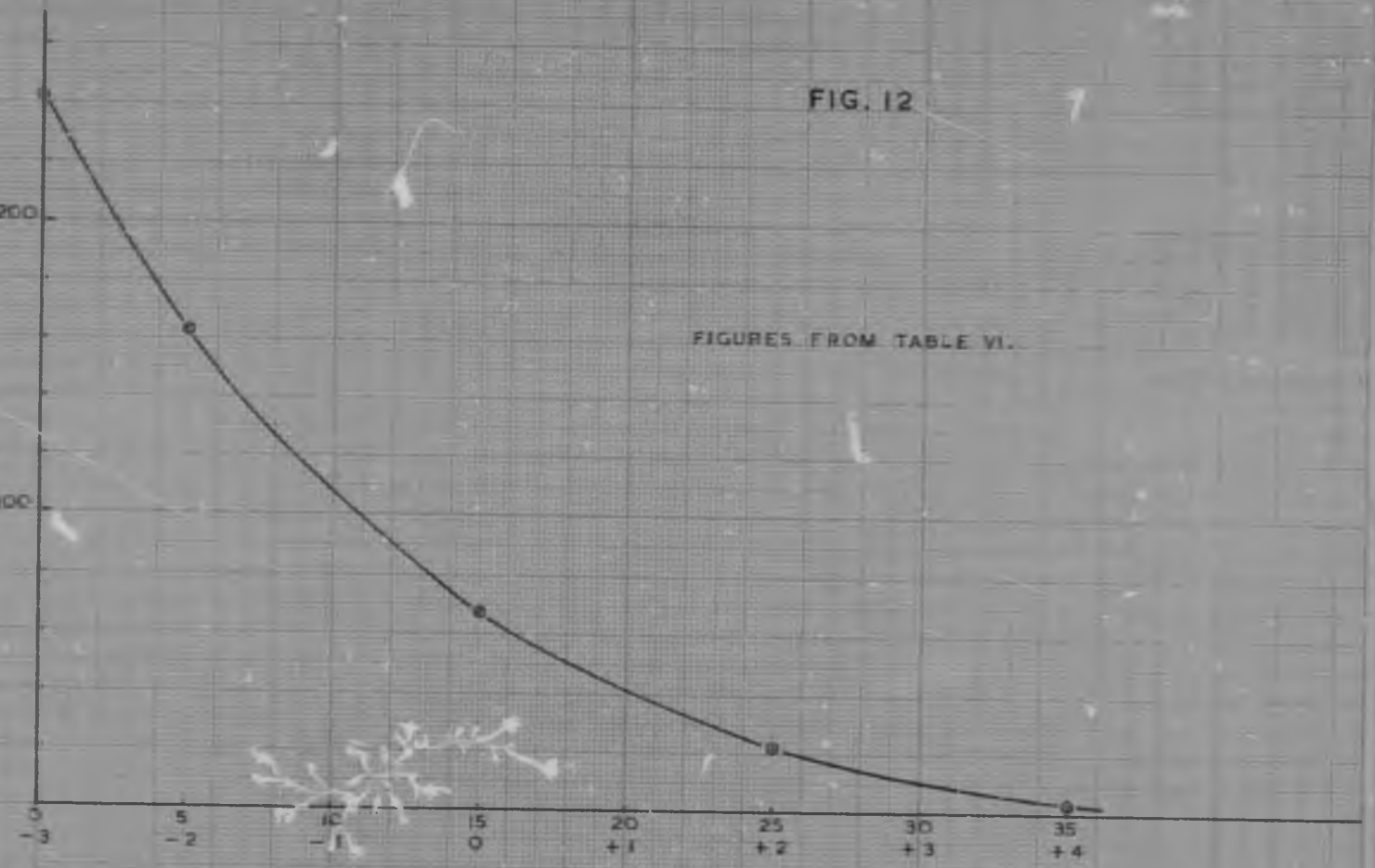
Our basic equation was the ogive

$$y = ne^{-cx^b} \qquad (11)$$

When taking intervals of unit distance, the t^{th} frequency moment is given by the equation (see Sichel, 1947):

$$J_t = \sum_{1}^{k} w_{x_1} (f_{x_1}^{t}) = \int_{x_1-\frac{1}{2}}^{x_k+\frac{1}{2}} y^t \, dx \qquad (12)$$

FIG. 12



FIGURES FROM TABLE VI.

in which the f_{x_1} are the observed frequencies; γ_{x_1} the weights attached to the observations; and k = the number of abscissal intervals.

As in our particular case k was (theoretically) infinite *) (page 59) and $x_1 = 0.5$ (page 49), we have, by virtue of (11) and (12):

$$J_1 = n \int_0^\infty e^{-cx^b} dx = \frac{n\Gamma(1/b+1)}{c^{1/b}} \qquad (13)$$

and

$$J_2 = n^2 \int_0^\infty e^{-2cx^b} dx = \frac{n^2 \Gamma(1/b+1)}{(2c)^{1/b}} \qquad (14)$$

As a result of the unequal intervals taken at the beginning of the curve, the evaluation of weights offered certain difficulties which were very elegantly solved by Mr Sichel in the following way.

If the point 15 on the abscissa of the graph (fig. 12, opposite) is taken as 0, the values x = 0, 5, 15, 25 and 35 become -3, -2, 0, +2 and +4 respectively. The ordinates are now indicated as y_{-3}, y_{-2}, y_0, y_2 and y_4 and a 3rd degree parabola of the formula

$$y = px^3 + qx^2 + rx + s \qquad (15)$$

can be fitted through the points x_{-3}, y_{-3}; x_{-2}, y_{-2}; x_0, y_0; x_2, y_2 and x_4, y_4 of the ogive. The area under the curve between x = -3 and x = -2 can then be found by integration, viz.

$$\int_{-3}^{-2} (px^3 + qx^2 + rx + s)dx = -\frac{65}{4}p + \frac{19}{3}q - \frac{5}{2}r + s \qquad (16)$$

Next p, q, r and s are to be expressed in terms of the ordinates, for which we obtain from (15) the following equations:

$$y_{-3} = -27p + 9q - 3r + s$$
$$y_{-2} = -8p + 4q - 2r + s$$
$$y_{+2} = 8p + 4q + 2r + s$$
$$y_0 = s$$

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*) Nota bene: the symbol k used here has naturally no relation to the parameter of same notation in equations (13), (15) and (16) on page 58.

Solving these equations for the constants p, q, r and s yields

$$p = \frac{1}{40}y_2 + \frac{1}{8}y_{-2} - \frac{1}{15}y_{-3} - \frac{1}{12}y_0$$

$$q = \frac{1}{8}y_2 + \frac{1}{8}y_{-2} - \frac{1}{4}y_0$$

$$r = \frac{3}{20}y_2 - \frac{3}{4}y_{-2} + \frac{4}{15}y_{-3} + \frac{1}{3}y_0$$

$$s = y_0$$

Substituting the above values into (16) we find

$$\text{Area} \Big|_{-3}^{-2} = \frac{1}{192}(40y_{-3} + 61y_{-2} - 6y_0 + y_{+2})$$

On application of Weddle's rule for approximate integration the area under the ogive is equal to

$$0.3(1y_5 + 5y_{15} + 1y_{25} + 6y_{35} + 1y_{45} + 5y_{55} + 1y_{65}) +$$

$$+ 0.3(1y_{65} + 5y_{75} + 1y_{85} + 6y_{95})$$

The area from x = 0 to x = 85 then becomes

$$0.3y_5 + 1.5y_{15} + 0.3y_{25} + 1.8y_{35} + 0.3y_{45} + 1.5y_{55} + 0.6y_{65} +$$

$$+ 1.5y_{75} + 0.3y_{85} + 1.8y_{95} + \frac{40}{192}y_0 + \frac{61}{192}y_5 - \frac{6}{192}y_{15} + \frac{1}{192}y_{25} =$$

$$= \frac{1}{960}(200y_0 + 593y_5 + 1410y_{15} + 293y_{25} + 1728y_{35} + 288y_{45} +$$

$$+ 1440y_{55} + 576y_{65} + 1440y_{75} + 288y_{85} \ldots\ldots)$$

The weights will thus be the respective coefficients of the different terms.

In steps the procedure of evaluating the expected frequencies ensues now:

1) determine $J_1 = \sum_1^k w_{x_1}(f_{x_1})$ and $J_2 = \sum_1^k w_{x_1}(f_{x_1}^2)$

2) solve equations (13) and (14) whence we obtain the following formulae for the parameters

$$b = -\frac{\log 2}{\log \frac{J_2}{nJ_1}} \qquad (17)$$

and

$$\log c = b(\log^n/J_1 + \log\Gamma(1/b + 1)) \qquad (18)$$

3) substitute the computed values for b and c together with the respective practical values of the variable x into (11) to find the y_1

4) taking differences yields the calculated f_1.

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