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Rothamsted Report for 1963

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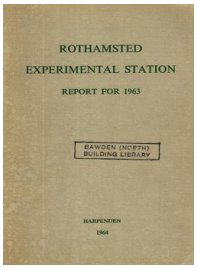

Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT F. G. W. JONES

A. G. Whitehead, C. D. Green, Diana M. Parrott and Patricia Cox were appointed to the staff, and H. R. Wallace left to become head of a new plant nematology laboratory in Adelaide, Australia. Dr. M. B. Harrison (Long Island, U.S.A.), Dr. G. O. Poinar (Riverside, U.S.A.) and Miss P. H. Yuen (Singapore) joined the department as temporary workers. Dr. G. N. Rao (Coimbatore, India) was awarded the Ph.D. of London University and D. J. Hooper became a Member of the Institute of Biology after submitting a thesis. In June the department sponsored a Symposium of the British Society for Parasitology at which related problems in animal and plant nematology were discussed. In September seven members of the staff attended the Society of European Nematologists' Symposium at Auchincruive, and in October and November the department helped with the nematology course at Imperial College Field Station, Sunninghill. A new glasshouse, header-house and soil-processing room were completed in December. The department now possesses adequate means of growing plants and handling soil.

Extraction of Nematodes from Soil

The clay fraction of a 200-ml sample of soil can be dispersed without injuring the nematodes present by adding 100 ml of 5% "Calgon" solution, 250 ml of distilled water and "vibro-mixing" for 5 minutes (sandy soils), 7 minutes (loams) or 15 minutes (most clay soils). This is followed by adding 1 litre of distilled water and "vibro-mixing" for a further 5 minutes. A simple, transparent sedimentation-tank has been developed for separating from the slurry thus produced vermiform nematodes, other than large Dorylaimoidea such as *Xiphinema* and *Longidorus*. In the tank the nematodes and fine soil particles are separated from the coarse soil particles; the nematodes are then separated from the fine particles by sieving and centrifuging. The method extracts about 70% of the nematodes in soil and gives results comparable with other methods. (Whitehead)

For the extraction of large Dorylaimoidea such as *Xiphinema*, 50-ml samples of soil are shaken with water in 1-litre conical flasks. After shaking the flask is filled to the brim, allowed to stand and organic matter that rises to the surface floated off. After further shaking the suspension is poured through 50- and 100-mesh sieves. In a test with *X. diversicaudatum* 79% of the nematodes recovered were on the 50-mesh and the remainder on the 100-mesh. Those passing the 50-mesh sieve were mainly small larvae, and none seemed to pass the 100-mesh sieve. The method recovered 76 ± 10% of nematodes added to soil and gave reasonably consistent results. (Larbey)

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Systematics and Bionomics

Various species of *Xiphinema*, *Longidorus* and *Paralongidorus* were found to have two nerve rings instead of the single ring present in most Dorylaims. A hemizonid was usually associated with the anterior ring and a hemizonion with the posterior. (Goodey and Hooper)

Populations of *Xiphinema index*, the vector of fan-leaf virus of grape vines, from Europe and California were very similar except for great variations in tail shape in some individuals. A new species of *Xiphinema*, close to *X. mammillatum*, *X. index* and *X. diversicaudatum*, found in England, France, Germany, Portugal and the U.S.A. around the roots of sugar cane, grape vines and other plants, is characterised by a curved front end, off-set lip region, equatorial vulva, opposed gonads and round tail ending in a mammillate peg. Males are rare. Very many *X. brevicolle* were found around roots of several plants from Israel and Portugal, and the same or a related species in soil from Venezuela and the U.S.A. (Lima and others)

The depth distribution of *Xiphinema diversicaudatum* was studied in Geescroft Wilderness. On the four sites sampled, few were found in the top 4 cm of soil. On two sites the shallow-rooting perennial plant dog's mercury (*Mercurialis perennis*) was abundant and most *Xiphinema* (60–70/50 ml soil) were in the root zone 4–8 cm down. On the other sites the deep-rooting tree, elm (*Ulmus campestris*) was abundant. Here there were only 10/50 ml soil at 4–16 cm, but numbers increased to 30–40/50 ml at 16–20 cm. Below 16–20 cm numbers were much the same at all sites and decreased gradually with increasing depth. The clay content of the soil increased from 30% at 10–20 cm, to 45% at 24–28 cm and 60% at 40 cm. Below 20 cm the finer texture of the soil and compaction may limit the movements of *X. diversicaudatum* to cracks and fissures. *X. diversicaudatum* is 65 μ in diameter, and therefore requires a labyrinth with channels greater than this diameter through which to move. Experiments on how the environment affects the vertical movement of *X. diversicaudatum* in the soil suggest that only under certain conditions can the nematode control its vertical movements. When the soil pores were much larger than the diameter of the nematode, although individuals did move both upwards and downwards, downward movement predominated, both when the pores were full of water and when the moisture film was adjusted to the conditions for maximum movement. In pores large enough for the nematode to pass through but small enough to resist lateral movements of its body, *X. diversicaudatum* moved downwards when the pores were full of water but upwards when the pore moisture favoured active movement. Gravity seems to have an overbearing influence on the movement of *X. diversicaudatum*, and the nematodes overcome this only when the pore size and moisture films allow them to produce maximum propulsive thrust. The influence of moisture gradients on movement seems secondary to the negative geotactic response. At optimum pore size and moisture content *X. diversicaudatum* moved upward with or against the moisture gradient. In an experiment in which gravity was nullified by continually reversing its direction the nematodes moved to the wetter end of a moisture gradient. (Larbey)

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The nematodes of regenerated woodland and ungrazed grassland were studied in Broadbalk Wilderness. The wooded section is mainly hawthorne, sycamore, ash and hazel, with brambles, dog's mercury and cuckoo pint as the dominant ground cover. The ungrazed section is coarse grasses and herbaceous plants. The composition of the sward is different at one end shaded by an oak tree. Thirty-one genera are common to both halves, but there are differences in species. For example, the only spiral nematode common to both areas is a new species of *Helicotylenchus*. Five other species of *Helicotylenchus* and three species of *Rotylenchus* occur in one or other of the habitats, but not in both. In the wooded section three species of *Helicotylenchus* occupy different areas. There is a similar zoning of species in the ungrazed section. The vertical distribution of different species also differs. The new species of *Helicotylenchus* mentioned above occurs commonly down to 18 cm, whereas a species of *Rotylenchus* is almost confined to the top 6 cm. (Yuen)

To improve our knowledge of the nematode fauna of agricultural land in East Anglia 165 fields were sampled after crops of spring barley, winter wheat, sugar beet and main-crop potatoes. The fields were on nine distinct soil types, and five fields were sampled for each soil–crop combination. Samples from 41 winter wheat fields were analysed and species of *Pratylenchus*, *Tylenchorhynchus* and "sharp-tailed" *Tylenchidae* were present in nearly every sample, spiral nematodes (Hoplolaimidae) in half and *Pratylenchus* in a third. At one site a new species, found about wheat roots, possesses affinities with the important root parasitic genera *Radopholus* and *Pratylenchus* and with *Pratylenchoides*. More specimens of this and similar species were later found about grass roots in several localities. (Whitehead)

Male and female *Panagrolaimus rigidus* were reared separately and then placed together on nutrient agar plates at differing temperatures. Eggs were not laid at 0° C, though copulation occurred, and more were laid at 20° C than at higher or lower temperatures. The worms were most active at 20° C, as indicated by the numbers that emerged from sand placed in test-tubes with the ends of the tubes in water at different temperatures. (Greet)

Root-knot Nematodes, *Meloidogyne* spp.

In the life cycles of species of root-knot nematodes sexual and asexual phases seem to alternate irregularly. Except with *Meloidogyne javanica*, males are frequent in old cultures on tomato roots raised originally from single larvae. The numbers of males in the egg sacs of root-knot nematodes differ from species to species and between different isolates of the same species. They also differ with the variety of host plant grown and the age of the root infestation. However, in tests with tomato plants in pots infested with an isolate of *M. javanica*, which produces relatively few males, or an isolate of an undescribed species, which produces many males, neither removing the plant tops nor inoculating plants of different ages significantly changed the numbers of males in the egg sacs.

A study of serial sections of unilocular galls produced on tomato roots by *M. javanica*, *M. incognita*, *M. arenaria*, *M. arenaria thamesi* and two

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other *M.* spp. showed that giant cells were always present and well developed near the head of egg-producing females. Histologically, galls produced by *M. hapla* differed somewhat from those induced by the six other species, all of which produced similar ones. Excessive lateral root development in the region of the galls, usually regarded as a characteristic of *M. hapla* infestation, also occurs to some extent with other *M.* spp. (Whitehead)

A new species of *Meloidogyne* native to Britain was found in samples collected by National Agricultural Advisory Service officers in Gloucester, Cornwall, Devon, Somerset, Shropshire and South Wales. Most samples were on barley or wheat, but ryegrass was infested in Wales and sugar beet in Shropshire. Common weeds growing among cereals were also attacked. The nematode caused patches of stunted plants. The root galls differ from those caused by most species of *Meloidogyne* in being small and elongated, and there is not the excessive lateral root development caused by *M. hapla*. Club-shaped galls are formed on root tips and, when other parts are attacked, the root may become hooked or spirally twisted. Galls on sugar beet were also small and situated on lateral roots, usually close to the main tap root. (Franklin)

Experiments show that the alien species of root-knot nematode, *M. incognita acrita* and *M. javanica*, which occur in glasshouses on tomatoes and cucumbers, cannot survive the winter outdoors in Britain; in glasshouses they survive for at least 7 months in soil without hosts. The false root-knot nematode, *Nacobbus serendipiticus*, an alien glasshouse species, has a life cycle of 57 days at 21° C. Like the species of root-knot nematode, its eggs hatch freely in water without the need of a special stimulus. (Clark)

Biological Races of Stem Eelworm, *Ditylenchus dipsaci*

To study the interrelationships of biological races of *Ditylenchus dipsaci*, stocks of lucerne, red clover, white clover, oat, narcissus and tulip races were propagated on their respective host plants. Pot experiments to compare the rate of population increase of the different races on several host and "non-host" plants showed that they reproduced in plants previously reported as resistant or "non-hosts". Thus, the oat race reproduced, but did not increase in numbers, on hyacinth, increased tenfold on tulip and about two hundredfold on oats. Red clover, white clover, lucerne and tulip races all increased rapidly on onion seedling.

Several races have been cultured aseptically on plant callus tissue in the dark at 24° C, and some callus tissues provide a suitable medium for mating individuals of different races. Red clover race and narcissus race reproduced but did not increase, tulip race increased threefold and lucerne race over tenfold on lucerne callus during 30 days. Both the narcissus and the tulip race increased fivefold and lucerne race fortyfold on red clover callus.

Monocotyledon callus was produced on a modified Heller's agar medium. By adding a trace of diaminoethanetetra-acetic acid and increasing the quantity of 2,4-dichlorophenoxyacetic acid (2,4-D) to 5 mg/1 onion formed callus, though it grew only slowly. Oats produced an actively

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growing callus on Heller's medium containing glucose instead of sucrose, 2 mg/1 indole acetic acid and 5 mg/1 2,4-D. (Webster)

Cyst-forming Nematodes *Heterodera* spp.

Biotypes. Attempts to study the interrelations of biotypes of the potato cyst-nematode, *Heterodera rostochiensis*, using potato callus tissue, failed. Callus of several potato varieties was produced on an auxin-fortified nutrient agar. A resistant potato hybrid derived from *S. tuberosum* ssp. *andigena* formed firm, golden callus that grew rapidly and subcultured well, but similar callus was not obtained from *S. vernei* or *S. multidissectum*. Aseptic larvae placed on sterile callus did not develop beyond the third stage. Pear-shaped, fourth-stage larvae excised from potato roots were surface sterilised and inoculated singly into callus. Some developed further and, after 2 weeks, a swollen female, a fully developed male and two males still within the larval cuticle were found. There was no evidence of tissue changes to suggest that they had fed on the callus, although it is assumed that the swollen female must have done so or have obtained nutrients from the culture medium. Callus tissue seems an unfavourable medium for culturing *H. rostochiensis*. (Webster)

Observations on the Cabbage Cyst-Nematode, *H. cruciferae*

A group of small cells in females of *H. cruciferae* resemble a similar group of large rectal, gland-cells in root-knot nematodes, *Meloidogyne* spp. and, like them, may be concerned in the formation of the egg sac. The egg sac starts to form soon after an immature female has ruptured the root cortex from anal and vulval secretions which come together. The mixture is viscous, whereas the anal secretion alone is fluid and readily spreads over the whole vulval cone and sometimes farther forwards. In water the two secretions appear as separate layers with slightly different refractive indices. Immature females with small egg sacs make slow dorso-ventral movements of both vulva and anus.

Second-stage larvae move actively over root surfaces and make frequent spear thrusts. Sometimes they enter the roots between cells where lateral roots emerge. A larva was filmed penetrating the end wall of an oblong epidermal cell. For 2 hours it slowly moved back and forth within the cell, pausing while its "head" was pressed against one or other end wall. The lip region moved slowly across the cell wall, causing it to bulge under the thrust, but the spear was seldom protruded. At first the larva divided its time between the two end walls, but later concentrated its attention on one of them and, with forwardly directed body waves, increased its thrust. Eventually, with head pressed into the corner of the junction of end and lateral walls, spear thrusting started; at first intermittently, then more quickly and persistently, directed across the end wall and back to the junction where rigidity was greatest. At first the spear bent with each thrust and the end wall resisted penetration by its flexibility. Periods of rest interrupted activity until the spear tip finally broke through at the junction of end and lateral walls. Spear thrusting was now continuously maintained

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and directed progressively across the end wall, each thrust breaking through. Suddenly the wall collapsed and the anterior quarter or third of the larva shot through, as its body straightened. It moved to the next end wall and continued thrusting its spear into one corner, until activity declined and the slow movement back and forth was resumed. Oesophageal bulb contractions were not seen while the cell wall was penetrated, and there was no obvious secretion from the salivary glands.

Once inside roots larvae move very slowly and become immobile except for movements of the head during feeding. After enlargement, females rupture the cortex and most lie with the dorsal side towards the root. The head of the adult female is surrounded by brown material, which may serve as a cement that ensures attachment to the head. The material seems not to arise from the excretory pore, which remains free of it. The heads of young larvae move freely in all directions, but the angle of movement is only 20°–30° in adult females.

All stages, except fourth-stage and adult males, have been seen feeding, and all behave similarly. A long period of intermittent spear thrusts at different angles is followed by short, quick thrusts with the spear protruding successively farther into a cell. The spear then remains fully protruded and still, usually for 1–5 minutes, while the oesophageal bulb makes spasmodic, local, muscular contractions. The pump in the bulb then pulsates several times, pauses and pulsates again. Pulsations may later be sustained. Although the contents of a much distended dorsal oesophageal gland duct of a third-stage male larva could be seen moving forward with each bulb "spasm", saliva was not seen to be expelled from the spear. (Doncaster)

Hatching factors. Another 4½ tons of potato-root diffusate were produced for the extraction of the natural factor for *H. rostochiensis* by the Biochemistry Department. Large raised beds of soil, planted with potatoes, were used instead of pot-grown plants, with no adverse effect on the potency of the root diffusate. The potatoes grew better than in pots, and the method saved labour. Protection from rain was unnecessary, as heavy showers or continuous rain barely drained through the soil. Sugar-beet root diffusate was produced by germinating seeds in bulk in inverted aspirator jars fitted with an automatic watering system, without the use of soil. The bioassay of fractions of root diffusates of potato and sugar beet continued, mostly by absorptiometric methods for the former and standard counting techniques for the latter. (Shepherd and Cox)

The amount of debris floated from soil during the extraction of *H. schachtii* cysts, which must not be dried, has been greatly lessened by washing for an hour on a 40-mesh sieve, which forces long pieces of debris through the holes. The cleaned float contains mostly spherical debris among the cysts, and the proportion is small enough for the pipetting method of Jones and Gander to be used, thus saving much labour. (Shepherd)

Synthetic hatching agents for beet cyst-nematode, *Heterodera schachtii*. About 350 substances were tested for their ability to stimulate hatching of the beet cyst-nematode. Fifteen per cent were very active, 35% intermediate and 50% inactive or inhibitory. In common with dyes already

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found active, most of the active compounds possess a conjugated unsaturated system able to undergo oxidation or reduction. This suggested there might be a correlation between hatching activity and redox potential. Out of 25 compounds of known redox potential, 12 were as active as a standard beet-root diffusate, 7 were less active and the others inactive. Phosphate buffer added to maintain pH 7·0 lessened activity. The pH of unbuffered solutions ranged from 6·0 to 7·0 except for Bindschedler's Green, which was pH 3·5. Changes of less than 0·5 units occurred in the pH of unbuffered solutions during the tests. Although the redox compounds tested covered a wide range, no correlation between redox potential and hatching was established. This is illustrated by the great activity of compounds of such widely different potentials as Bindschedler's Green (+0·224 V) and phenosafranine (—0·252 V), by the inactivity of *o*-bromophenolindo-2,6-dibromophenol (+0·239 V) and by the only moderate activity of methyl viologen (+0·432 V) and benzyl viologen (—0·320 V). The hatching tests are not made in bacteriologically sterile conditions and this may obscure certain factors, for the redox potential *in vivo* may differ from that measured by E'_0.

Other active compounds include organic acids, amines, lactones, pyridine derivatives and other heterocycles, ketones, phenols, quinones, various thioureas and a few inorganic compounds; these groups also contain many inactive compounds. No activity was found among the alcohols or esters, and only very slight activity among the aldehydes and amino acids tested. With one exception, simple unsubstituted aliphatic mono- and dibasic carboxylic acids were inactive. Active acids were in four groups: (*a*) aromatic acids with the carboxy group directly attached to the aromatic ring; (*b*) β-keto acids; (*c*) αβ-unsaturated acids; and (*d*) α-hydroxy aliphatic acids, but not all members of these groups were active. Twelve out of 16 quinones and 17 out of 24 phenols were active. The inactive quinones were unsubstituted *p*-quinones. Active phenols were either substituted or unsubstituted, and activity was strongly influenced by the position and nature of the substituent groups, which applied also to other aromatic compounds tested.

Some of the active compounds may act directly, but others may undergo an initial change either outside or within the cyst, egg or larva before stimulating hatch. (Shepherd and Clarke, Biochemistry Department)

The hatching of the beet cyst-nematode in some synthetic hatching agents was studied in detail. Hatching in acetone and *o*-bromophenolindophenol was similar to that in root diffusate, in tolylene blue more eggs hatched and hatched sooner than in diffusate, and picric acid and *o*-aminobenzoic acid greatly accelerated hatching. In picric acid larvae emerged from cysts at an average rate of one per cyst per 20 minutes from the 2nd to 6th day of the test, and hatching was almost complete by the 10th day, when 80% of the eggs had hatched. In a standard root diffusate eggs were still hatching slowly after 40 days.

Stimulus by the natural hatching factor for *H. schachtii* in beet-root diffusate need be applied for only 1½ hours for some effect to be noticeable on the subsequent hatch of eggs in water. Sixteen hours in root diffusate followed by a week in water gave hatches similar to continuous immersion

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in diffusate for a week, and 8 hours was almost as good. This means that experiments can be done in the absence of root diffusate, provided an initial stimulus is applied.

When highly active compounds were applied to soil containing cysts some were more efficient than beet diffusate in emptying the cysts, and none was phytotoxic at the concentrations used. (Shepherd)

The pea and oat cyst-nematodes (*H. goettingiana* and *H. avenae*) differ from most other cyst-nematodes in that eggs cannot be stimulated to hatch by root diffusates or any known artificial agents *in vitro*. Pea cyst-nematode, however, is stimulated in soil in which host roots are growing. Experiments suggest that failure to hatch *in vitro* is not caused by extracting cysts from soil, because eggs in extracted cysts were stimulated to hatch when they were placed in pots of sand or soil sown with peas. In field plots at Woburn evidence was obtained that encysted eggs of cereal cyst-nematode were also stimulated to hatch by host roots, but less than those of *H. goettingiana* or *H. rostochiensis* when their respective host plants (pea and potato) were grown. (Shepherd)

Soil Sterilants

Tests of efficacy. In a co-operative experiment with Mr. A. L. Winfield and Dr. A. F. G. Slootweg, yields of daffodils and tulips, grown in replicated plots on Norfolk silt infested with *Ditylenchus dipsaci*, were not increased by treating the soil with "D-D", metham-sodium and dazomet, even though there was an increase in soil ammonia-nitrogen. It was not possible to estimate kill of *D. dipsaci*. (Peachey and Gasser)

Dibromochloropropane ("Nemagon") drenched on florists' roses grown in pots under glass killed the plants at doses as small as 0·5 ml active material per plant; drenches of 0·25 ml active material per plant did not kill roses growing in the open. (Peachey)

In a Cheshunt glasshouse "D-D" at 800 lb/acre was applied to soil infested with *Xiphinema diversicaudatum* in early December. Apart from two small areas, the treated soil was steamed one month later and a small test bed of roses was planted in early March when the soil smelled strongly of "D-D". These grew well, and the remaining area was planted in late April. *X. diversicaudatum* were not found in soil treated with "D-D". The roses grew better on land steamed after fumigation than on land only fumigated. (Peachey and Mr. E. B. Brown, National Agricultural Advisory Service, Cambridge)

Moistened potato-root eelworm cysts (*Heterodera rostochiensis*) were exposed to vapours of TD 183 (tetrachlorothiophene), UC 17956 (a chlorinated heterocyclic compound) and to standard commercial soil sterilants. The experimental compounds were nematicidal, but their small vapour pressures (less than 1 mm Hg at 21° C) are likely to prevent their adequate dispersion in British soils. (Peachey)

In plots at Woburn treated with methyl bromide, dazomet, methyl isothiocyanate and "D-D", the kills of potato-root eelworm estimated from soil cores taken 8–12 in. deep were similar to those obtained from the top 8 in. Metham-sodium killed only half as many as others in the 8–12-in.

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layer. At Rothamsted, however, metham-sodium was equally effective at all depths. (Peachey, Rao and Buahin)

Estimation techniques. The kill of potato cyst-nematode by methyl bromide, metham-sodium, dazomet, methyl isothiocyanate and "D-D" in plots at Woburn was measured by counting: (*a*) live eggs left unstained after immersion of cysts in New blue R stain; (*b*) numbers of larvae hatched from cysts immersed in potato root diffusate for 6 weeks; (*c*) numbers of larvae recovered after maceration of potato and tomato roots grown in pots of field soil, or sterilised soil inoculated with cysts, for 6 weeks; and (*d*) numbers of new cysts formed in pots of field soil or sterilised soil inoculated with cysts in which potato plants had been grown for 15 weeks. Estimates of live eggs and larvae in roots of plants grown in field soil were the most accurate and convenient. Estimates of kill from the numbers of larvae in roots of indicator plants did not differ significantly when done at different temperatures (14°, 19° or 33° C) or when plants were allowed to grow for 3 or 9 weeks instead of 6. All techniques showed that methyl bromide killed most eelworms. These tests of technique confirm results of similar tests on glasshouse soils.

Potato plants were grown in pots of sterilised soil inoculated with potato cyst-nematode larvae. When inoculation was near planting time more larvae invaded the roots and developed into cysts than when inoculation was done 14 days before or 28 days after planting. Numbers of larvae in roots or cysts produced were related to the number of larvae in the inoculum when the inoculum was small, but not when it was large. The growth of the potato plants was related to the severity of eelworm attack. (Rao and Peachey)

Application techniques. A soil injector built by the National Institute of Agricultural Engineering and tested at Rothamsted injects a sterilant under pressure at points from 6 to 12 in. apart and 6–12 in. deep, and is powered by a Landmaster L150. To overcome traction resistance of up to 1,000 lb, a winch is mounted on the front and anchored to the far end of the plot. The machine is being tested in glasshouses and fields.

Fluorescent pigments dissolved in soil fumigants were rapidly absorbed when they came into contact with soil particles and did not disperse with the fumigant. Those used made it easier to trace the admixture of sterilant dusts with the soil, even with very small percentages of tracer.

Because soil sterilants are corrosive and irritant, rubber gloves are usually worn when handling them. Gloves made from rubber (for domestic or industrial use), neoprene and polythene were immersed in: (*a*) a 40% solution of methyl isothiocyanate; (*b*) "D-D"; and (*c*) chloropicrin. All sooner or later were penetrated by the sterilants. Pieces of glove were sealed into vessels so that sterilants were on one side of the material and air on the other. The air was passed through a condenser to liquefy traces of the sterilant vapour that penetrated the material. Gas chromatographic analyses done on the condensate from sterilant (*a*) contained greater concentrations of the toxic constituent than the original formulation. With domestic rubber gloves the increase was threefold. (Peachey, Greet and Lord)

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