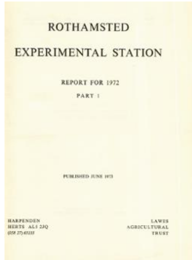
ROTHAMSTED RESEARCH

Report for 1972 - Part 1

Full Table of Content


ROTHAMSTED

EXPERIMENTAL STATION

REPORT FOR 1972

PART 1

Nematology Department

F. G. W. Jones

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NEMATOLOGY DEPARTMENT

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The Nematology Department studies nematodes or eelworms, minute worm-like creatures that damage all parts of plants, although roots are the organs most frequently attacked. By identifying new species, studying their host ranges and behaviour, and by trying to understand how the bodies of economically important species function, the Department adds to knowledge of these creatures which may ultimately lead to new or better ways of controlling them. The Department also attempts control of harmful species with nematicides on our own and on other farms. Although nematicides often increase yields, this alone is not enough. It is important that systems of control, which may include crop rotation, planting resistant varieties and the application of nematicides, should leave fewer nematodes in the soil than were present initially.

Effects of root endoparasitic nematodes on plant growth

In an experiment at Woburn which tested the effects of irrigation and fumigation on the growth of the eelworm-resistant potato variety Maris Piper, and susceptible Pentland Dell, observations were made on growth of potato plants infested with the potato cyst-nematodes, *Heterodera rostochiensis* or *H. pallida*. The treatments applied over six years produced widely different nematode population densities. Tops and roots of both potato varieties were sampled periodically during the growing season. The numbers of nematodes in the soil before planting, and of larvae that invaded the roots subsequently, were estimated. Roots and tops were weighed, leaf-areas calculated, and the major nutrients they contained analysed. The nematodes invading the roots influenced all the above. The potassium, phosphorus, magnesium and nitrogen content of the plants on 22 June was least in plants with the greatest number of nematodes in their roots, whereas the calcium content was greatest. Nitrogen was the nutrient least affected by nematode attack, but uptake of potassium was markedly decreased.

Measurements of the amount of water in leaves (water potential) of heavily and lightly infested plants and the opening of the stomata, which control water loss (measured by resistance to the passage of air) showed that the effects of nematodes on the size of plants can be largely accounted for by changes in stomatal resistance to air flow (see p. 39). As potassium ions have a regulatory function in the operation of the guard cells that open and close the stomata, nematodes probably cause water stress in two ways, by reducing the size of the root system and by depriving the plant of potassium ions, which are essential for the plant's efficient use of water. (Evans, Trudgill and Parrott, with Parkinson, Physics Department)

In the same experiment at Woburn, the effects of nematode infestation on the ability of roots to exploit the soil were studied. The distribution of roots down to 70 cm was followed in soil cores 12 weeks after planting and the length and weight of main and lateral roots recorded. When many nematodes were present in Pentland Dell or Maris Piper, root systems were small and did not penetrate much below plough depth (50 cm below the crests of ridges), but Maris Piper root systems were twice the weight of those from Pentland Dell. So, as the mean diameter of Maris Piper roots was smaller than that of Pentland Dell roots, weight for weight Maris Piper roots are longer and may penetrate better. They may also afford less internal space per unit length and thus may provide

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poorer sites for the development of female nematodes. In both varieties, infestation decreased the length of lateral roots. The distribution of roots at each depth studied was fairly uniform along and across the ridges which made it possible to calculate the volume of soil exploited per plant (Table 1). When the volume exploited was plotted

TABLE 1

Volumes of soil exploited per plant ($m^3 \times 10^{-2}$) assuming that nutrients move to roots over a distance of 0·5 cm

	Pentland Dell		Maris Piper		
Population size	Small	Large	Small	Large	Large*
Depth					
Ridge	1·8	0·7	2·7	2·7	1·1
Ploughed layer	2·1	0·1	3·0	1·5	1·3
Subsoil	0·6	0·0	1·0	0·1	0·3
Total	4·5	0·8	6·7	4·3	2·7
Yield of tubers/plant (g)	1071	200	1424	977	815

* *H. pallida*, all other populations *H. rostochiensis*

against the yield of tubers, the best straight line fit was obtained if it was assumed that nutrients moved from 0·5 to 1·0 cm. (Evans, Trudgill, Thompson, and Pandé)

Feeding and behaviour

Selection of feeding sites by tylenchid nematodes. Films of 11 species of Tylenchida suggest a common pattern of exploratory behaviour leading to feeding in which simple direct responses to a succession of specific stimuli are made or not, according to physiological state. Following an initial stimulus, nematodes sense gradients of chemical attractant, and test the structure and deformability of surfaces encountered. It is possible to define the activity of a larva cutting its way out of the egg, and larvae and adults penetrating and feeding on their hosts, in terms of simple sequences of behaviour. In cutting behaviour, for example, the cutting of a slit through an egg shell or plant-cell wall, through which the nematode then moves, consists of confluent, successive punctures made by the stylet, each successive perforation being positioned by localised exploratory behaviour. In feeding as opposed to cutting, successful perforation is followed in some species by the emission of saliva and subsequently by the cessation of emission and the pumping action of the median bulb; in others saliva mixes with the ingested food. (Doncaster and Seymour)

Mechanics of feeding. To enable the internal pressure of plant parasitic nematodes to be recorded directly, methods of making cannulae to penetrate or hold small nematodes in an observation cell were developed. A multi-channel pen recorder and pressure-gauge transducers, with minute volume displacement, recorded pressure changes via a fine glass cannula with great sensitivity in less than a tenth of a second. The mechanics of stylet action in penetrating cells are little known. Stylet protractor muscles are universally present, but there is no mention in the literature of stylet retractor muscles in tylenchs. Films of stylet protraction and retraction in *Heterodera*, *Aphelenchoides* and *Ditylenchus* spp. show stylet action in hatching and feeding, and suggest that the stylet is withdrawn by an elastic recoil mechanism involving the protractor muscles, the anterior body wall muscles and cuticle. In studying the mechanics of penetration, the following are being taken into account: the wall ultrastructure, the fineness ratio (length divided by greatest

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width), taper and length of stylet, the mechanical properties of the nematode body wall, the force available for penetration and the area over which it is applied, the speed and rate of stylet thrusts, and the depth of penetration. Protractor muscles of the stylets of male *Heterodera rostochiensis* exert a force of about 0·1 dynes., equivalent to over 500 kg/cm^2 at the stylet tip. Stylets are of the right size to penetrate a plant cell wall by thrusting apart the cellulose fibres of the wall, rather than by cutting individual fibres. (Seymour)

Feeding of *Thornenema wickeni*. Adults of *T. wickeni* seem to have no passage connecting pharynx and intestine (*Rothamsted Report for 1969*, Part 1, p. 177). Under the light microscope, juveniles appear to have a passage but one cannot be demonstrated in sections under the electron microscope. Nevertheless electron micrographs of juveniles and adults show the intestines contain organic detritus, fibrous material and many organisms that may be symbionts.

T. wickeni populations are difficult to culture in observation chambers or to observe feeding but in water agar at pH 7 to 8·5 they survive two weeks at 20°C. None of the usual culture media was suitable for them, nor did they feed on organic detritus, plant roots, diatoms, protozoa or crustaceans (Harpacticidae) extracted with *T. wickeni* from fen soil. In *T. wickeni* freshly extracted from peat or kept alive in the laboratory, the intestine was well filled with brown, organic matter. Although dilute suspensions of colloidal graphite or Indian ink are ingested by rhabditid nematodes, they soon killed *T. wickeni*. Occasionally, on water agar surfaces, freshly extracted *T. wickeni* pumped with the pharynx and drew in air bubbles, which seems the first record of a stylet-bearing nematode ingesting air. (Doncaster and Shepherd)

Observations on *Laimaphelenchus penardi*. Although *Laimaphelenchus* spp. are generally regarded as predaceous nematodes, two populations of *L. penardi* extracted from yellow lichen reproduced slowly on agar plate cultures of the fungus *Botrytis cinerea*. Progeny from the cultures placed on agar plates with many *Aphelenchoides blastophthorus* or *Caenorhabditis* spp. made no attempt to attack or feed on them. These *Laimaphelenchus* populations seem to reproduce parthenogenetically because they contain no males and sperm was not seen in the females. *Laimaphelenchus* spp. have four characteristic caudal tubercles, each expanded and fringed at its distal end. Studies with the scanning electron microscope show that in *L. penardi* the fringe consists of a single rosette of eight to ten finger-like processes at the end of each tubercle. The processes are about 1 μm long, 0·2 μm in diameter and radiate from the longitudinal axis of the tubercle at its tip. In active adults and larvae the tail is curled ventrally, particles become attached to the tubercles, which seem not to move independently, and attached nematodes have difficulty in getting the tail free. However, the tubercles are probably not suckers, for nematodes do not adhere to glass fibres as do some plectids that have a caudal sucker. It is assumed that the curled tail and fringed tubercles anchor specimens in their natural habitat. (Hooper and Stone)

Defaecation. Because pressures in the bodies of nematodes are high, when the anus opens during defaecation intestinal contents are forcibly expelled. Tylenchid nematodes examined appear to have a prerectum and prerectal valve which limits the quantity of faeces voided, but fixed specimens of *Aphelenchoides blastophthorus* showed no definite prerectal region or valve. However, observations on live specimens showed that a part of the intestine behaves as a prerectum and that a prerectal valve in the intestinal wall separates it from the intestine proper. So, during defaecation, only the contents of the prerectum and rectum are voided. A backward wave of relaxation and contraction of the

https://doi.org/10.23637/ERADOC-1-127

muscles in the body wall fills the prerectum with faeces, the prerectal valve closes and then the faeces are ejected; the rectal valve and anus operate independently by their own muscles. (Seymour and Doncaster)

Mate finding. All plant parasitic nematodes have a soil phase when they must move through the labyrinth of soil spaces to find host plants or mates. The relative simplicity of nematode sensory receptors, the fact that those in pairs on the head or tail are very close together, and the nature of the environment, preclude direct attraction. To study the mechanisms involved in host or mate finding, the attraction of male cyst-nematodes to their females was chosen because single females on agar are a well-defined source of attractant and the attractant is specific. A system based on roots, which emit many substances and have regions different in morphology and physiological state, or on a particulate medium are too complicated for initial studies.

Tracks made by male cyst-nematodes when finding their females were recorded and hypotheses developed to explain them. A computer program was then made, based on these hypotheses, and the tracks drawn by the computer were compared with real ones made on agar plates. The computer model showed that, when the paired sense organs situated close together on the head of a nematode are assumed to act as a single organ which adapts to a rise in concentration of the attractant, three main parameters govern the form of the track. The first is an innate tendency of individual males to deviate to one side or the other, the second the direction and degree of turning when the nematode moves forward again after stopping, and the third the rate the sense organ adapts to the attractant. The rate of adaptation is also related to the rate of forward movement under stimulation. There was an optimum rate of deviation from straight-line movement forwards which depended on the distance of the male from the source of attraction. Approach to the female was slower when deviation was in the direction away from her position. Also large deviations from straight line movement greatly slowed the approach. Small deviations also slowed the approach and lessened the influence of the direction of deviation on events. Approach to the source of stimulus was faster when the head of the model turned through 60° rather than 45° after stopping. Too rapid adaptation led to an insensitive response in shallow gradients of the attractant in which the model male circled the source with frequent stops and restarts. When adaptation was too slow, the male went past the source and wasted time returning.

An interesting feature essential to the model, which agrees with observed behaviour, is that the male is assumed to have a 'clutch' mechanism situated between a quarter and a third of the length of the body behind the head. Normally, in free forward movement, the nematode body oscillates to produce a waveform that moves backwards, providing propulsion. When a nematode stops (e.g. when it discerns a fall in the concentration of the attractant), the 'clutch' is 'disengaged' and the head swings in arcs but the wave motion is not transmitted backwards and only when an increased concentration is perceived is the clutch engaged and forward movement in a new direction resumed. Several features of behaviour still require analysis so that the model can be refined and the tracks drawn by computer males made to resemble more closely those made by real ones. (Green with Beasley and Jackson, Computer Department)

Electron microscopy

Spermatogenesis in *H. rostochiensis*. In the development and structure of sperm, studied in thin sections under the transmission electron microscope, tylenchoid nematodes seem to differ from rhabditid and aphelenchoid nematodes. In males of *H. rostochiensis* which do not feed when adult and survive free in soil for about two weeks, the early stages of

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spermatogenesis, including all cell divisions occur in the pre-adult stage. So, the male has a limited sperm output conserved by the copulatory spicules which are an efficient intromittent organ. The spermatogonia, at the tip of the larval testis, are cells containing ribosomes, Golgi apparatus, and an oval nucleus with a membrane and a prominent nucleolus. They proliferate by mitosis and later by meiosis producing primary and secondary spermatocytes and spermatids. The spermatocytes become amoeboid and are closely packed so that their irregular outlines fit together like parts of a jigsaw-puzzle. In the adult male these stages disappear and the cells nearest the testis tip become spermatids with two distinct parts. The spermatid proper is amoeboid, has a nucleus without a membrane and contains mitochondria and many bundles of fine fibrils scattered through the cytoplasm. The other part of the cell lacks a nucleus but contains many ribosomes and Golgi vesicles and is evidently nutritive. The two parts are joined by a narrow isthmus which is pinched off some distance along the testis, when the nutritive part is discarded and engulfed by the cells of the testis wall. Spermatids and spermatozoa have an electron-dense outer membrane and a fine inner plasma-membrane lined with microtubules in parallel arrays over the whole inner surface, which spiral along the pseudopodia to their tips. In tangential section the system of microtubules resembles a fingerprint. During development from spermatocyte to spermatozoa the chromatin of the nucleus changes form at least five times. Sometimes it is in electron-dense clumps, when it appears to be homogeneous and sometimes it is in thin strands and sometimes in thick ones. Throughout spermatogenesis the nuclear membrane is lacking.

On entering the female the sperm remain amoeboid and stay in the spermatheca or move between the oviduct wall and the oocyte. Entry of the sperm into an oocyte was not observed but several sperm were seen with pusedopodia adhering close to the oocyte membrane, which was discontinuous in the region of contact. (Shepherd, Clark and Kempton)

Structure of copulatory spicules. The paired copulatory spicules of *Heterodera* males are sclerotised structures inserted in the vulva of the female during copulation that provide a channel through which the sperm are transferred. The shape of the spicule tip has been used to sub-divide the genus into the subgenera *Heterodera* (*Heterodera*), in which females are lemon-shaped and the male spicules have bi- or possibly tridentate tips, and *Heterodera* (*Globodera*), with round females and the male spicules with simple tips. The spicules of six species from the first group and five from the second were examined under the Stereoscan electron microscope. In *Heterodera* (*Heterodera*) all spicule tips were straight and bidentate whereas in *Heterodera* (*Globodera*) they were curved and had a single point. In all species from both groups there were two small pores near the tip of each spicule situated in the slight hollow between the cusps of *Heterodera* (*Heterodera*) and on the curved portion just behind the tip of *Heterodera* (*Globodera*).

Spicules of *H. schachtii* and *H. rostochiensis* were sectioned and examined under the transmission electron microscope. In both the spicule is a hollow rod with a slightly swollen proximal end, a cylindrical shaft for about a quarter of the spicule length, which flattens distally into a blade with incurved edges and a pointed tip. The whole structure is curved outwards laterally and downwards ventrally. When retracted, the spicule tips with their pores just project from the cuticular collar surrounding the cloacal aperture. When extended, the spicules fit snugly inside the collar, their curvature causing the proximal ends to move towards each other. The dorsal wings of the two spicules interlock and their ventral wings overlap forming a complete tube. When inserted into the vulva of the female, the tips splay apart and hold the male in position while the tube formed by the spicules ensures that the sperm are transferred without spillage.

The proximal end of the spicule encloses a core of nerve tissue, which originates from

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the lateral nerve cord and can be traced to the spicule tips, where two nerve processes end just beneath the two pore openings. Presumably the pores open into sense organs which may play a part in copulation. It is difficult to observe mating in *Heterodera* as the female is large and opaque and, in lemon-shaped species, the vulva is obscured by a gelatinous egg sac. As the female is sedentary the male must actively seek out the vulva. Although the spicules are obviously sensitive organs, their role in copulation is not fully understood. Whether the complex cuticular pattern around the female vulva has any guiding function is also unknown. (Clark, Shepherd and Kempton)

Shapes of larval heads in the genus *Heterodera*. Scanning electron microscopy shows that second stage larvae of *Heterodera* spp. have a smooth-surfaced area of tissue surrounding the stomal aperture, the oral disc, bounded laterally by the two lateral lips, which carry the amphidial apertures, and dorsally and ventrally by the two pairs of sub-lateral lips. The lips are, in turn, surrounded by the first head annule. This arrangement is seen in *Heterodera* (*Globodera*), whereas in *Heterodera* (*Heterodera*), the oral disc is elongated dorso-ventrally and the two pairs of sub-lateral lips are usually more or less fused. The form of the disc and lips in species with lemon-shaped females is of several different types. Arranging the species according to type of lip pattern produces sub-groupings which divide further some of the accepted species groupings within the sub-genus based on characters of the vulva. So far, larvae of 35 species have been examined and seven groups distinguished.

The surface morphology of *Heterodera* larval heads under the scanning electron microscope shows lip structures more clearly than do heads mounted vertically under the light microscope. The same is true for other species of Tylenchida examined and scanning electron microscopy will probably reveal differences of taxonomic value in groups other than *Heterodera*. (Stone and Course)

Taxonomy and biology of round-cyst nematodes

***H. pallida*, a new species of potato cyst-nematode.** Pathotypes of *Heterodera rostochiensis* with white or cream females have been recognised as different in several ways from those with golden females (*Rothamsted Report for 1970*, Part 1, 149; *for 1971*, Part 1, 165). These differences are as great as or greater than those between other *Heterodera* (*Globodera*) species or between some *Heterodera* (*Heterodera*) species such as *H. cruciferae* and *H. carotae*. So, these pathotypes have been described as a new species, *H. pallida*. Besides the colour differences between females, *H. pallida* differs from *H. rostochiensis* in a number of morphological details and in the pattern of bands produced by electrophoresis of soluble proteins in polyacrylamide gels.

H. pallida occurs in the United Kingdom, Europe, the Faroes, South America, India and New Zealand and probably elsewhere. Mixed infestations with *H. rostochiensis* are not uncommon especially in the U.K. *H. pallida* seems to have originated in upland areas of Latin America on indigenous solanaceous hosts together with *H. rostochiensis* and other *Globodera* species parasitising Solanaceae and has been disseminated with potato tubers and by commerce first to Europe and then to other parts of the world. Both species of potato cyst-nematode have pathotypes defined by their ability to multiply on hybrids with resistance genes derived from cultivated and wild *Solanum* spp. British pathotype A is *H. rostochiensis*: British pathotypes B and E are *H. pallida*. Dutch pathotypes B and C are *H. rostochiensis*: Dutch pathotype D is *H. pallida*. Dutch A and British A are identical and so are Dutch D and British E. Altogether three pathotypes of *H. rostochiensis* and two, possibly three, pathotypes of *H. pallida* are known but more may be found when new sources of resistance and new populations of nematode are tested.

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Because the British and Continental pathotyping schemes are based on tests done on different lines of resistant plants, some confusion exists. There is need for a standardised international nomenclature, able to accommodate the different sources of resistance favoured in different countries and to discard any that fall into disuse. The nomenclature must now recognise that there are two species. At a meeting in June held in Münster, West Germany, joint tests were proposed to establish a standardised nomenclature throughout Europe. (Stone and Course)

Size variations of *Heterodera rostochiensis* larvae. Using larval dimensions to distinguish *Heterodera* species makes the assumption that size is uninfluenced by external factors. To test this assumption, individual plants of *Solanum tuberosum* cv. Arran Banner and ten other species of tuberous *Solanum* grown in pots of sterile soil were each inoculated with *H. rostochiensis* British pathotype A raised on Arran Banner. After 16 weeks the cysts produced were extracted and ten randomly selected new cysts from each host were measured after being artificially hatched, heat relaxed and fixed in 4% formaldehyde solution (Table 2). Using Spearman's rank correlation test no association was found between the average size of females (cysts) produced on the different hosts and the average length of larvae from them ($r = -0{\cdot}3$). Nor was there any correlation between the mean number of cysts produced on each host species and larval size ($r = 0{\cdot}1$) but too few new cysts were produced on all hosts to make the test meaningful. The length of the stylet varied as much between individuals from the same host as between those from different hosts. These results support the assumption that the dimensions of second stage larvae of *Heterodera* are little influenced by the nutrition of the parent nematode. (Stone and Course)

TABLE 2

Means and standard deviations of cyst and larval dimensions of Heterodera rostochiensis *from 10* Solanum *spp.*

	Cysts ($n = 10$)	Larvae ($n = 25$)	
	Mean diameter (length excluding neck + width/2) (μm)	Body length (μm)	Stylet length (μm)
S. tuberosum cv. Arran Banner	566±55	473±19	21·7±0·7
S. brevidens	547±73	455±26	21·6±1·3
S. cardiophyllum	631±71	434±29	22·8±0·8
S. columbianum	548±60	455±31	21·8±1·1
S. dulcamara	541±116	446±40	21·8±0·7
S. hjertingii	512±121	473±33	21·2±1·4
S. microdontum	590±80	461±26	21·6±1·1
S. multidissectum	618±63	428±29	21·7±1·2
S. papita	605±68	457±35	21·8±1·5
S. sucrense	609±46	453±36	22·1±0·5
S. verrucosum	512±67	445±26	21·4±1·1

Soluble proteins of round-cyst and other nematodes. Although the patterns obtained from electrophoresis of negatively charged, water-soluble proteins in polyacrylamide gels have recently been used to distinguish races and species of round cyst-nematodes, little is known of the identity of the proteins. In an attempt to characterise them further, the gels were tested for selected enzymes which, it was reasonable to suppose, would be present, and to see where they occurred along the gels.

https://doi.org/10.23637/ERADOC-1-127

Results with proteins extracted from *Heterodera rostochiensis* (pathotype A) and *H. pallida* (pathotype E), *H. solanacearum*, *H. mexicana* and *H. virginiae* indicate each band contains not one but several different substances. There is also some evidence, as yet unconfirmed, that host plants influence the electrophoretic patterns. (Greet)

Comparable work on several species of the vermiform nematodes, including aphelenchoids, *Longidorus* and *Ditylenchus* nematodes, showed there were marked differences between the protein patterns of different genera but differences between species within genera tended to correspond with the degree of difference indicated by the traditional methods of taxonomic appraisal. Therefore without refinement the technique does not seem helpful in separating closely allied species. The protein pattern from *Aphelenchoides cibolensis* was similar to that from *A. composticola* whereas those of *A. rutgersi* and *A. sacchari* were different from both but similar to each other. *A. blastophthorus* and a related undescribed species gave patterns different from each other and from the other *Aphelenchoides* spp. tested. The protein pattern from a population of *Aphelenchus avenae* without males was very different from another population with males, suggesting they were perhaps different species. Larvae of *Bursaphelenchus fungivorus*, *Paraphelencus pseudoparietinus*; *P. myceliophthorus* had patterns similar to those of their adults but all three species had patterns different from all the other Aphelenchoids tested. Large and small forms of *Longidorus leptocephalus* had the same patterns which were very close to those of *L. elongatus*, whereas *L. macrosoma* had a pattern very different from both. The 'giant-race' of *D. dipsaci*, which is tetraploid and the oat race which is diploid, both obtained from field-bean (*Vicia faba*) stems, had different protein patterns. (Hooper, Pike and Trudgill)

Appearance of *Heterodera pallida* in plots at Woburn Farm. Although resistant potatoes were grown in the same plots in Long Mead, Woburn, for ten years without evidence of a change of cyst-nematode population from *H. rostochiensis* (pathotype A) to *H. pallida* (pathotype E), in another field nearby, *H. pallida* had increased noticeably in one plot where Maris Piper potatoes had been grown continuously for five years. Increases in the number of eggs in the soil of other similar plots suggested that they too contained *H. pallida*, and this was confirmed by pot tests. These revealed that *H. pallida* was well established in plots neighbouring that where it was first seen. Fumigation with 'DD' for the first three years and dazomet for the next two delayed but did not prevent *H. pallida* from establishing itself where the variety Maris Piper was planted.

That *H. pallida* reached detection level at about the same time in most plots growing Maris Piper continuously suggests it was thinly but evenly distributed over the whole area before the experiment started in 1966, with perhaps a larger infestation on the plot where it appeared first. Many years ago the field in which this experiment is located contained allotment gardens and so different parts had different histories. Other possibilities are that *H. pallida* was introduced and widely dispersed on Maris Piper seed potatoes or spread by machinery from the plot where it was first observed. The apparent absence of *H. pallida* on a few plots in one half of the experiment is difficult to explain.

The time taken, about five years, for *H. pallida* to increase to detectable numbers, in plots apparently uninfested initially is much the same as that required by *H. rostochiensis* on similar soil on another field at Woburn. (Parrott, Berry and Matthews)

Inheritance of the ability to overcome resistance. Single male, single female crosses made in 1970 showed that a Bolivian population and a British pathotype A population of *H. rostochiensis* interbred freely (*Rothamsted Report for 1970*, Part 1, 153). Larvae of the Bolivian population are able to become female in the roots of potato, var. Maris Piper, which possesses resistance gene H_1 whereas larvae of British pathotype A populations

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(Woburn, Feltwell) all become males. To determine the inheritance of this ability to become female (and therefore to reproduce) on plants with gene H_1, bulk matings were made between these populations by adding males to roots of potted potato plants bearing only virgin females. The F_1 progeny were tested on Arran Banner (susceptible) and Maris Piper (resistant) as were the F_2 progenies from females that developed on both hosts.

The F_1 progeny of the crosses reproduced poorly on Maris Piper (Table 3) and supported the hypothesis that ability to multiply on plants with resistance gene H_1 is recessive. If it were a simple single-gene mechanism, the numbers of F_2 females expected on Maris Piper should be a quarter (25%) of those on Arran Banner whether the F_1 host was Arran Banner or Maris Piper. The observed percentages suggest other factors are involved. These could be modifying genes, which would explain the 4% of females on Maris Piper in the F_1 generation compared with the 1% for the Woburn and Feltwell selfings. The ratio of numbers expected in F_2 could also be changed by a shift in the sex ratio or by competition between the genotypes. (Parrott, Berry and Matthews)

TABLE 3

Development of Heterodera rostochiensis *females on potatoes with gene H_1 from* Solanum andigena *by F_1 and F_2 progeny of crosses between populations able (Bolivia) and unable to multiply on plants with gene H_1*

Parental populations		F_1 generation		F_2 generation			
				F_1 host Arran Banner		F_1 host Maris Piper	
Females	Males	No. of females on Arran Banner	†% females on Maris Piper	No. of females on Arran Banner	†% females on Maris Piper	No. of females on Arran Banner	†% females on Maris Piper
Woburn	Woburn	161	1	245	4	—	‡
Feltwell	Feltwell	76	1				
Bolivia	Bolivia	66*	56	101	156	236	99
Bolivia	Bolivia	120	65				
Woburn	Bolivia	236	3	109	50	136	18
Feltwell	Bolivia	147	6				
Bolivia	Woburn	227	4	138	36	165	39
Bolivia	Feltwell	135	3				

* One plant only; all other tests two or more replicates
† No. on Arran Banner = 100
‡ Total hatch from F_1 cysts was 700 larvae; these were inoculated on to one Maris Piper plant and produced four females

Potato cyst nematode hatching factors. One of the hatching factors present in potato root extracts (eclepin 1) was chosen for detailed study and isolated in microgram amounts as an acidic, non-crystalline gum. Attempts were made to convert it into a derivative sufficiently volatile for gas chromatography which could be unambiguously identified by regeneration of the starting material. In most attempts, the reaction products failed to initiate hatching after appropriate treatments. In others only the starting material was recovered. It was found that the acidic group was resistant to esterification by the usual methods. However, in several experiments the sequence, hatching factor–derivative–hatching factor was achieved. Yields from the process were small and a complication was that, in thin layer chromatography of the reaction mixture, four separate compounds were detected which, after treatment, caused hatching. Possibly the four products include compounds in which derivative formation is incomplete. The compound which travelled

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furthest on thin layer chromatograms was assumed to be the least polar and, when inserted in a gas chromatograph, produced five significant peaks. A fraction splitting device was used to collect materials from the peaks which were hydrolysed to return them to their original state. The hatching capabilities of these materials are now being assessed. (Clarke and Hennessy)

Trials with nematicides

Control of pea cyst-nematode. At Gosbeck, E. Suffolk, on clay loam soil heavily infested with pea cyst-nematode, *H. goettingiana*, aldicarb, 'Du Pont 1410' (5-methyl 1-(dimethyl-carbamoyl-N-[(methylcarbamoyl)oxy]thioformimidate) and 'Ciba-Geigy 10576' prevented the nematodes harming or multiplying on pea roots (Table 4) and greatly increased yields of shelled peas. None of the treatments prevented pods from being infested with caterpillars of the pea moth (*Ernarmonia nigricana*). (Whitehead, Tite, Fraser and French)

TABLE 4

Effect of aldicarb, 'Du Pont 1410' and 'Ciba-Geigy 10576' on yields of peas var. Dik Trom and multiplication of pea cyst-nematode

Treatment	Amount a.i. (kg/ha)	Yield of shelled peas (t/ha) Fresh	Dry	Multiplication of pea cyst-nematode
Untreated	0	7·0	2·7	×3·0
aldicarb	5·6	11·8***	4·5***	×0·9
	11·2	13·0***	5·0***	×0·6
	22·4	12·4***	4·7***	×0·8
'Du Pont 1410'	5·6	11·9***	4·6***	×0·6
	11·2	12·8***	5·0***	×0·6
	22·4	13·8***	5·2***	×1·2
'Ciba-Geigy 10576'	5·6	9·4**	3·6**	×0·6
	11·2	11·6***	4·5***	×0·6
	22·4	12·6***	4·9***	×0·7

, * Significantly more than untreated at 1%, 0·1% probability, respectively

Control of potato cyst-nematodes. Field experiments since 1968 show that potato cyst-nematodes (*H. rostochiensis* and *H. pallida*) can be controlled and the yield of susceptible potatoes greatly increased in infested soils by incorporating small amounts of aldicarb or 'Du Pont 1410' in the topsoil just before potatoes are planted. In 1972 the effectiveness of less dusty granules of aldicarb and 'Du Pont 1410' and of granules containing a new organophosphorus nematicide ('Ciba-Geigy 10576') were tested on sandy loam heavily infested with *H. rostochiensis* at Woburn Experimental Farm. The nematode was controlled by all three chemicals and yields of Pentland Crown potatoes greatly increased. Aldicarb and 'Du Pont 1410' were rather more effective than 'Ciba-Geigy 10576' (Table 5). In infested, irrigated peaty loam at the Arthur Rickwood Experimental Husbandry Farm, Isle of Ely, the same formulations of aldicarb and 'Du Pont 1410' increased the yield of King Edward potatoes as much as did a dusty formulation of aldicarb and a liquid formulation of 'Du Pont 1410' (Table 6). These results suggest that the new, less dusty and hence safer granules are as effective in controlling *H. rostochiensis* as the older dusty ones.

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TABLE 5

Effect of aldicarb, 'Du Pont 1410' and 'Ciba-Geigy 10576' on yield of potatoes var. Pentland Crown and multiplication of potato cyst-nematode

Treatment	Amount a.i. (kg/ha)	Yield of tubers over 3·8 cm diam. (t/ha)	Multiplication of potato cyst-nematode
Untreated	0	18·8	×4·6
aldicarb	2·8	28·7***	×0·4
	5·6	28·1***	×0·5
	11·2	32·4***	×0·3
'Du Pont 1410'	2·8	32·7***	×0·5
	5·6	32·7***	×0·3
	11·2	30·3***	×0·5
'Ciba-Geigy 10576'	2·8	26·6***	×0·6
	5·6	26·0***	×0·5
	11·2	25·2**	×0·6

, * Significantly more than untreated at 1%, 0·1% probability, respectively

TABLE 6

Effect of different formulations of aldicarb and 'Du Pont 1410' on yield of potatoes var. King Edward in irrigated peaty loam infested with potato cyst-nematode

Treatment	Amount a.i. (kg/ha)	Yield of tubers over 3·8 cm diam. (t/ha)
Untreated	0	45·3
aldicarb 10G BC	8·4	62·1***
aldicarb 10G	8·4	55·5*
'Du Pont 1410' (24% liquid)	8·4	56·3*
'Du Pont 1410' (10% granules)	8·4	58·0**

*, **, *** Significantly more than untreated at 5%, 1%, 0·1% probability, respectively

H. rostochiensis is often abundant in peaty loam soil below 20 cm, where it may injure potato roots and so lessen potato yields. An experiment was made to study the effect on potato yields and on nematode control of incorporating 'Du Pont 1410', dazomet and 'Nemacur P' in moderately infested topsoil to a plough depth of 30 cm. This was done by treating the topsoil after, and before and after ploughing deeply with a digger plough to invert the furrow slices. 'Du Pont 1410' is water soluble and at low soil temperatures remains effective against the nematode for several months during which it would be expected to leach downwards, so the first treatments were applied in December 1971 before ploughing. Treating the topsoil with 'Du Pont 1410' or dazomet before ploughing and with 'Du Pont 1410' after ploughing in spring 1972 greatly increased the yield of King Edward potatoes in irrigated and unirrigated land (Table 7). The only spring treatment which significantly increased yield was 5·6 kg 'Du Pont 1410'/ha in irrigated plots. Controlling the nematode to plough depth therefore seems beneficial. Two equal doses of 'Du Pont 1410' or 'Nemacur P', one applied before ploughing in winter, the other in spring, controlled the nematode in soil to 20 cm deep as well as an equivalent amount applied in spring.

An experiment in Butt Close, Woburn (sandy loam), and Longmead, Woburn (sandy clay) tested control of *H. rostochiensis* by different amounts of formalin, a cheap and readily available compound, applied either as a drench or incorporated neat in the topsoil by rotary cultivation. Only the largest amount of formalin applied significantly increased the yield of Pentland Crown potatoes in both soils but even the largest amount failed to

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TABLE 7

Effect on yield of potatoes var. King Edward when peaty loam with potato cyst-nematodes, is treated with nematicides in winter and spring or in spring only

				Unirrigated		Irrigated	
Winter 1971	a.i. (kg/ha)	Spring 1972	a.i. (kg/ha)	Tubers (t/ha)	Multiplication	Tubers (t/ha)	Multiplication
Untreated	0	Untreated	0	40·2	×20	45·3	×26
'Du Pont 1410'	5·6	'Du Pont 1410'	5·6	44·6	×3	53·6*	×3
	5·6		11·2	50·8*	×1	55·4*	×2
dazomet	224		5·6	51·0*	×2	54·9*	×2
	224		11·2	48·4*	×1	60·2**	×2
Untreated	0		5·6	46·8	×8	55·1*	×8
	0		11·2	47·3	×3	51·3	×4
dazomet†	448	Untreated	0	48·1*	×6	49·9	×6
dazomet†(s)	448		0	44·7	×4	49·5	×9
Untreated	0	'Ciba-Geigy 10576'	5·6	43·8	×13	47·9	×13
	0		11·2	46·9	×22	43·6	×6
'Nemacur P'	5·6	'Nemacur P'	5·6	44·5	×3	51·7	×9
	5·6		11·2	44·2	×2	56·6*	×3
Untreated	0		5·6	50·2*	×7	43·8	×8
Untreated	0		11·2	44·8	×4	51·2	×5
dazomet	224		5·6	47·0	×10	55·6*	×6
	224		11·2	46·4	×2	52·4	×4
			Mean	46·1		51·3	

*, ** Significantly more than untreated at 5%, 1% probability, respectively (vertical comparison only)

† Half before, half after ploughing, (s) plots covered with Polythene sheeting until spring

TABLE 8

Effect of formalin and dazomet on yield of potatoes var. Pentland Crown and multiplication of potato cyst-nematode

		Sandy clay		Sandy loam	
Treatment	Amount a.i. (kg/ha)	Tubers over 3·8 cm diam. (t/ha)	Multiplication of potato cyst-nematode	Tubers over 3·8 cm diam. (t/ha)	Multiplication of potato cyst nematode
Untreated	0	16·4	×3·9	9·4	×1·0
dazomet	336	27·5	×5·9	24·8**	×1·1
formalin (40%)	500	25·8	×10·1	10·9	×1·3
	1000	24·7	×7·9	11·2	×1·0
	2000	32·4*	×4·2	18·8*	×1·2
formalin (0·9%)	500	19·8	×8·4	10·1	×1·6
(1·8%)	1000	23·1	×9·1	9·4	×1·0
(3·6%)	2000	29·2*	×9·2	14·5	×1·2

*, ** Significantly more than untreated at 5%, 1% probability, respectively

decrease the number of eggs after harvest (Table 8). In another experiment in Longmead, dazomet greatly increased the yield of Pentland Crown potatoes in heavily infested soil where extra FYM or fertilisers had no effect (Table 9).

An experiment to control the nematode, by a combination of nematicides, crop rotation and a resistant potato variety (Maris Piper) was started in 1972 on sandy loam in Stackyard Field, Woburn Experimental Farm. The rotations are resistant potatoes, sugar beet, barley, susceptible potatoes and susceptible potatoes, sugar beet, barley, susceptible potatoes. *H. rostochiensis* (pathotype A) numbers were controlled by Maris Piper potatoes, 'Du Pont 1410', and large amounts of dazomet incorporated in the topsoil,

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TABLE 9

Effect of dazomet and extra manures on yield of potatoes var. Pentland Crown and multiplication of potato cyst-nematode. Tubers over 3·8 cm diam. (t/ha)

	0	FYM *±1·7	Peat	†P_2O_5 + K_2O	Means	
Nil	15·7	19·7	17·1	18·7	17·7	±0·9
dazomet 336 kg/ha	35·0	34·8	34·6	38·6	35·8	
Means	25·4	27·3	25·9	28·7		
		±1·2				

* SE for vertical comparisons only
† 172 kg/ha P_2O_5 and 433 kg/ha K_2O, equivalent to amounts in FYM

half before and half after ploughing and by a combined treatment of dazomet and 'Telone' applied after ploughing in December 1971 (Table 10). Although 'Du Pont 1410' greatly increased the yield of the susceptible variety Pentland Crown it did not increase the yield of resistant Maris Piper, even though it prevented injury by potato cyst-nematodes. It may be that 'Du Pont 1410' is phytotoxic to this variety. (Whitehead, Tite, Fraser and French)

TABLE 10

Yield of two potato varieties and multiplication of potato cyst-nematode in soil treated with different amounts of three nematicides

		Maris Piper		Pentland Crown	
Treatment	Amount (kg/ha)	Tubers over 3·8 cm diam. (t/ha)	Multiplication of potato cyst-nematode	Tubers over 3·8 cm diam. (t/ha)	Multiplication of potato cyst-nematode
Untreated	0	22·0	×0·6	18·1	×3·6
dazomet (2)	224	26·9*	×0·5	25·4**	×1·6
(2)	336	30·9***	×0·5	31·1***	×0·6
(2)	448	32·6***	×0·6	34·6***	×0·8
(2)	672	33·4***	×0·5	35·2***	×1·0
'Telone' (1)	448	27·1*	×0·4	24·3**	×2·7
dazomet (1) and 'Telone' (1)	224 224	31·6***	×0·4	30·7***	×1·1
'Du Pont 1410' (S.)	5·6	20·2	×0·3	27·4***	×0·3
'Du Pont 1410' (S.) and 'Telone' (1)	5·6 224	25·2	×0·3	27·8***	×0·5
	Mean	27·8		28·3	

(1) applied after ploughing (2) half before, half after ploughing (S.) incorporated in topsoil in spring just before potatoes planted
*, **, *** Significantly more than untreated at 5%, 1%, 0·1% probability, respectively

Statistical analysis of 13 field experiments showed that yield of potatoes susceptible to *H. rostochiensis* was related inversely to the number of larvae in the roots of potted Arran Banner potato plants grown in soil from plots after treatment with different amounts of different nematicides. (Whitehead, with Dunwoody, Statistics Department)

Chemical control of stem nematode. In peaty loam (Methwold Fen, Norfolk), sandy loam (Wereham, Norfolk) and clay loam (Great Field II, Rothamsted Experimental Farm) treating onion rows at sowing time with granules containing aldicarb or 'Du Pont 1410' prevented much damage to onions by stem nematodes (*Ditylenchus dipsaci*) (Table 11). In the sandy loam and peaty loam soils 'Du Pont 1410' applied at sowing time

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TABLE 11

Effect of aldicarb, 'Du Pont 1410' and 'Ciba-Geigy 10576' on yield of onions var. Robusta and control of Ditylenchus dipsaci

Treatment	Amount a.i. (kg/ha)	Yield of onions (t/ha)	% wt of rotten onions in store	*D. dipsaci*/l soil from onion rows after harvest (log $(x + 1)$)
		Peaty loam		
Untreated	0	3·7	63	3·87
aldicarb (in onion rows)	1·4	17·2	51	3·58
	2·8	31·1**	23	2·62
	5·6	27·2**	15	1·74**
'Du Pont 1410' (in onion rows)	1·3	5·7	52	3·20
	2·6	19·0	24	2·83
	5·2	27·6**	30	1·96**
'Du Pont 1410' (broadcast)	2·0	5·1	80	3·58
	4·0	13·2	42	2·95
	8·0	26·3**	24	2·60
		Sandy loam		
Untreated	0	11·5	80	2·53
aldicarb (in onion rows)	1·4	33·1***	19	0·89
	2·8	27·7**	17	0·94
	5·6	34·0***	9	1·86
'Du Pont 1410' (in onion rows)	1·3	23·4*	31	2·70
	2·6	30·1**	24	1·57
	5·2	35·6***	25	0·91
'Du Pont 1410' (broadcast)	2·0	29·4**	17	0·88
	4·0	29·7**	27	3·10
	8·0	31·6***	14	0·81
		Clay		
Untreated	0	31·8	82	3·47
aldicarb (in onion rows)	1·4	42·3**	70	2·84
	2·8	39·3*	81	3·03
	5·6	39·3*	82	2·99
'Du Pont 1410' (in onion rows)	1·3	39·3*	86	2·74
	2·6	41·7**	83	2·73
	5·2	40·5**	82	2·96
'Ciba-Geigy 10576' (in onion rows)	1·3	31·5	86	3·45
	2·6	41·7**	72	3·19
	5·2	34·2	79	2·48

*, **, *** Significantly greater or less than untreated at 5%, 1%, 0·1% probability, respectively

increased yields more when it was incorporated in the onion rows than when it was broadcast and incorporated in the topsoil. 2·6 kg 'Ciba-Geigy 10576' (organophosphorus compound)/ha in the onion rows also increased the yield of onions in the clay loam soil but 5·2 kg/ha did not and is presumed phytotoxic. None of the treatments prevented stem nematodes multiplying in the crop. 5·6 kg aldicarb or 'Du Pont 1410'/ha lessened nematode multiplication in the peaty loam soil but not enough to control the nematodes completely which in some plots multiplied up to a thousand times between planting and harvest. (Whitehead, Tite and Fraser)

Stem eelworm on field beans. All except two field-bean sites at Rothamsted and Woburn were infested with stem eelworm, *Ditylenchus dipsaci*. The most heavily infested experimental site was in Warren Field, Woburn, and there were also badly infested patches on Great Knott III and Summerdells at Rothamsted. Both the 'oat-race' and the more devastating 'giant-race' of *D. dipsaci* now occur on most field-bean sites. The 'giant-race'

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was apparently introduced to some new sites this year with seed saved from an infested site on the farm last year. The 'giant-race' affected the tops of plants more than in previous years and caused severe internodal distortion and swelling, and necrosis of leaf petioles causing premature leaf fall; also pods were small and distorted and often contained infested seed. Of various soil sterilant treatments applied to some experimental sites, aldicarb was the only one that controlled *D. dipsaci* and increased yield by some 4 cwt/acre (see p. 144). (Hooper and Pike)

Control of migratory nematodes in barley. In a nematicide trial on sandy soil over Bunter pebble beds in Nottinghamshire, 'DD' was hand injected at 448 kg/ha and dazomet was rotavated in at 336 kg/ha in autumn, and aldicarb rotavated in at 1·73 kg a.i./ha in spring before planting. Nematode counts in May showed that aldicarb was the most effective nematicide and 'DD' the least (Table 12). In 'DD' treated plots the growth

TABLE 12

Effect of nematicides on nematode numbers in May and on growth of barley in June and July

	Untreated	aldicarb	'DD'	dazomet
Pratylenchus/g root	28	2*	12	2*
Pratylenchus/litre soil	519	19*	98*	10*
Parasitic nematodes/litre soil	3055	281*	1183	1459
All nematodes/litre soil	10260	1928*	10020	10650*
Tiller length, mm, June	64	68	51*	70
July	782	758	705*	789

* Significantly different from untreated at 5% probability

of barley measured by tiller length in June and July was less even than that of untreated plots, suggesting that 'DD' residues were phytotoxic. (Corbett and Webb)

Docking disorder of sugar beet. In large-scale experiments in five fields in West Norfolk infested with needle nematode (*Longidorus attenuatus*) and stubby-root nematodes (*Trichodorus* spp.), the effect of fumigating the row positions on sugar-beet yield was again tested. For the third successive year fumigating the rows before sowing sugar-beet seeds in them did not harm the seedlings. In two of the five trials it significantly increased sugar yield, although, in 1972, the irregular stunting characteristic of Docking disorder did not occur in any trial. (Whitehead and Tite)

Bromine residues after methyl bromide fumigation. In soil where *H. rostochiensis* and *Verticillium dahliae* are present, fumigation with methyl bromide increases potato yields in the year of fumigation and in the following year (*Rothamsted Report for 1970*, Part 1, 154), but bromine is absorbed by the crop. X-ray fluorescence spectrometry showed that there was more bromine in potato haulm than in tubers, that the amounts of bromine in tubers were small, and most was in the peel (Table 13).

FAO recommends that wheat grain should contain less than 50 ppm bromine. Bromine residues were detected up to three years after fumigating soil with methyl bromide. Most bromine was present when wheat followed a potato crop grown in fumigated soil, but it was less than 50 ppm. The bromine content of the grain decreased as the time between planting and fumigating increased (Table 14). Methyl bromide fumigation had no effect on wheat yields on these trial sites. (Corbett with Hide, Plant Pathology Department, and Brown, Pedology Department)

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TABLE 13

Bromine (ppm dry matter) in potato haulm and tubers in the year when methyl bromide was applied

	Haulm	Tubers	Peeled tubers	Peelings
Unfumigated	50	3	—	—
Methyl bromide 488 kg/ha	4000	168	—	—
Methyl bromide 975 kg/ha	6675	281	77	430

TABLE 14

Bromine content (ppm dry matter) and yields of wheat grain in 1972 in soil fumigated with methyl bromide at 975 kg/ha in previous years

	Unfumigated	Year of fumigation 1971	1970	1969
Bromine content	1	43	16	4
Yield (t/ha)	7·5	7·1	7·6	7·3

Oxime carbamate nematicides. Although the invasion of roots of potato plants grown in pots of soil mixed with granules containing the nematicide aldicarb followed the same pattern as in untreated soil and the numbers in the roots reached a maximum after five weeks, the numbers invading the roots were many fewer. When granules were added at a rate equivalent to 1·68 kg/ha incorporated to a depth of 15 cm in the field, the number of larvae decreased to 3·5% and at a rate equivalent to 11·2 kg/ha were fewer than 1%. Similar doses applied in field trials were much less effective and many more larvae entered the roots, suggesting that in agricultural practice the granules are poorly distributed in the soil. (Bromilow and Mr. P. H. Pritchard, Trent Polytechnic)

When 16·8 kg/ha of aldicarb in water was sprayed on to a grass sward at Bull Field, Woburn, on 3 March 1972, the initial dose was equivalent to 11 ppm in the top 10 cm of soil. In samples taken subsequently aldicarb itself was found in trace amounts in the soil surface only at the first sampling date. Its derivatives, aldicarb sulphoxide and sulphone were as in Table 15. By 18 July, in the last sample taken, only minute traces of sulphone remained. Aldicarb sulphoxide had a half life of about two weeks and underwent oxidation to the sulphone and degradation to non-toxic products. This agrees with the findings of Andrawes *et al.* (*Journal of Agricultural and Food Chemistry* (1971) **19**, 727–730) who showed that only a small part of the loss from soil was due to uptake by

TABLE 15

Persistence and leaching of aldicarb sulphoxide and sulphone* in sandy soil*

Soil sample depth in cm	14 March 1972 aldicarb sulphoxide	14 March 1972 aldicarb sulphone	28 March 1972 aldicarb sulphoxide	28 March 1972 aldicarb sulphone	2 May 1972 aldicarb sulphoxide	2 May 1972 aldicarb sulphone
0–10	3·55	0·45	1·78	0·47	0·040	0·16
10–20	0·44	0·107	0·44	0·14	0·022	N.D.
20–30	0·11	0·032	0·13	0·030	0·022	N.D.
30–40	0·063	N.D.**	0·087	N.D.	N.D.	N.D.

* Expressed as ppm of aldicarb sulphone
** N.D. none detected, less than 0·02 ppm present

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the crop. Although there was some leaching which was probably fastest for the sulphoxide, even after two months most of the toxic residues were in the top 10 cm of soil. (Bromilow and Trudgill)

A method was developed of determining trace amounts of the nematicide 'Du Pont 1410' and so of studying its behaviour in crops and soils to ensure a negligible residue in food crops. The nematicide is extracted from the soil or homogenised crop samples with ethyl acetate and interfering co-extractives removed by column or thin-layer chromatography. The carbamate group in the molecule is hydrolysed by 1*N* NaOH to yield the corresponding oxime, which is then methylated with dimethyl sulphate. This derivative is extracted and dissolved in a small volume of ethyl acetate, and assayed by gas–liquid chromatography on a column of 5% SE30 silicone gum on 'Chromosorb W' using a flame-photometric detector operated in the sulphur mode. The method is sensitive to about 0·02 ppm of 'Du Pont 1410' based on a 20 g sample. (Bromilow)

Appearance of 'scorch' in an experiment with sterilants and nitrogen against *H. avenae* on spring wheat. In an experiment testing the residual and cumulative effects of aldicarb, dazomet and formalin, on the growth and yield of spring wheat on land at Woburn infested with *H. avenae*, the crop grew normally until June. Thereafter the foliage became severely scorched and was also attacked by mildew (*Erysiphe graminis*) and *Fusarium* sp. The cause of the condition known as 'scorch' (see *Rothamsted Report for 1964*, 65) is unknown and its appearance reversed the yield trends in grain that might have been anticipated from the growth of plants (fresh top weights) in May (Table 16). Straw

TABLE 16

Spring wheat var. Kleiber, formalin, dazomet, and aldicarb ('Temik') Woburn, 1972

		Formalin		dazomet		aldicarb		
Kg N/ha	Untreated	Res.	Cum.	Res.	Cum.	Res.	Cum.	Means
		Fresh top weights/15 plants (g) May 1972						
63	42·7	41·5	47·1	39·9	41·2	45·0	48·2	43·7
126	58·3	58·5	64·6	55·4	65·3	63·4	75·6	63·0
189	60·0	73·8	70·2	74·0	71·6	75·5	82·3	72·5
Means	53·6	57·9	60·6	56·4	59·4	61·3	68·7	59·7
		Grain yields (85% D.M.) t/ha						
63	1·58	1·74	2·03	1·84	1·95	2·32	3·49	2·13
126	1·34	1·27	1·27	1·28	1·51	1·79	3·11	1·66
189	1·28	1·32	1·27	1·03	1·47	1·42	1·51	1·33
Means	1·40	1·44	1·52	1·38	1·64	1·84	2·71	1·71

yields were similarly diminished. The best yield followed aldicarb and the smallest rate of nitrogen fertiliser, the worst followed residual dazomet and the largest rate of nitrogen fertiliser. Although the stunting and whitening of the ears and the rolling of the flag leaves of the wheat plants was worst on plots that received most nitrogen, the amounts of nitrogen in affected and unaffected plants were similar and there was no correlation between severity of 'scorch' and the invasion of seminal roots by larvae of the cereal cyst-nematode *H. avenae*.

For the second year in succession the plots treated with aldicarb outyielded those treated with formalin or dazomet. The last two chemicals have previously given much improved yields in other cereal experiments at Woburn. By far the best control of

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H. avenae followed the second application of aldicarb, although the population of plots that received aldicarb in the previous year only, increased from 2 eggs/g air-dried soil at the end of 1971 to 12 eggs/g at the end of 1972, i.e. to the same numbers as in the control plots. (Williams and Beane, with Williams, Chemistry Department)

Formalin and pathogens of wheat. By the last year of an experiment in which formalin had been applied as a drench to control pathogens of wheat, the following formalin sequences had been applied:

1970	1971	1972
—	—	—
—	f	—
f	—	F
f	f	F

In 1972 formalin was applied in autumn or in spring to plots subsequently sown with spring wheat variety Kolibri. Formalin was also applied in autumn to plots subsequently sown with winter wheat, variety Maris Ranger, in autumn and in spring. Nitrogen was applied to the experiment at four rates, 75, 125, 176 and 226 kg/ha respectively. Yields and post-harvest soil populations of *H. avenae* are in Table 17. The egg counts in parentheses are those for 1971. A striking result throughout this experiment has been the poor

TABLE 17

Spring/winter wheat and spring and autumn formalin applications, Woburn 1972

	Sown	†Formalin applied	Formalin sequence - - -	- f -	f - F	f f F	Mean
Winter wheat							
t/ha	Autumn	Autumn	2·08	1·85	3·85	2·73	2·63
eggs/g soil			1·4	1·1	1·6	2·2	1·6
			(1·3)	(7·3)	(6·1)	(2·5)	(4·3)
t/ha	Spring	Autumn	1·48	1·55	1·85	1·71	1·65
eggs/g soil			4·2	4·2	8·0	7·7	6·0
			(3·7)	(10·8)	(6·8)	(14·0)	(8·8)
Spring wheat	Spring	Autumn					
t/ha			1·75	1·50	1·89	1·65	1·70
eggs/g soil			8·6	10·1	13·7	12·9	11·3
			(9·6)	(12·7)	(5·4)	(3·9)	(7·9)
t/ha	Spring	Spring	1·60	1·62	1·51	1·47	1·55
eggs/g soil			13·1	11·8	13·9	11·2	12·5
			(13·2)	(12·1)	(26·5)	(10·7)	(15·6)

† –, no formalin; f, formalin in earlier years; F, formalin in 1972

multiplication of *H. avenae* on winter wheat which is usually a good host. The best yield responses to formalin applied for the 1972 crop were from the plots sown with winter wheat in autumn. Visual assessments of the root invasion by *H. avenae* larvae ('knotting') agreed well with the number of larvae that invaded the seminal roots. Although the cereal cyst-nematode injury was worse in spring wheat, formalin, whether applied in spring or autumn had little effect on yield.

Experiments from 1964 onwards show that formalin at Woburn often causes unusually large increases in *H. avenae* populations. This may be because formalin removes an unknown microbial inhibitor or enemy. So, samples of soil taken in February, April and May from treated and untreated plots were studied. Subsamples were mixed, crushed and

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dilutions in distilled water added to plates of nutrient and potato dextrose agar. Aureomycin was added to the latter to suppress bacteria. After incubation at 20–25°C no significant differences appeared in bacterial colonisation of the nutrient agar plates. Plates made in February from formalin-treated soil bore no fungal growths but there were many colonies (mainly *Trichoderma* and *Penicillium* spp.) on plates from untreated soils. No differences in the kinds of genera were observed in later samples but the numbers of colonies differed very considerably until five months after the November 1971 formalin application. The genera found were: *Cephalosporium*, *Chaetomium*, *Dichlaena*, *Epicoccum*, *Mortierella*, *Penicillium*, *Phoma*, *Pyrenochaeta*, *Trichoderma* and *Truncatella*. Although there was no evidence to connect any of these commonly occurring soil fungi with a decrease in nematode invasion or development, the observations show that formalin had a marked effect on the abundance of certain genera for as long as five months after application. As the potato dextrose agar medium with aureomycin is only one of many possible media used to detect soil fungi, other media might detect formalin sensitive fungi which could inhibit *H. avenae* multiplication. (Williams, Beane, with Salt, Plant Pathology Department and Walker, Soil Microbiology Department)

Miscellaneous observations

Reproduction of *Aphelenchoides blastophthorus* and *A.* sp. on *Botrytis cinerea*. Although *A. blastophthorus* was originally described as feeding within Scabious buds, it readily reproduces on agar plate cultures of the fungus *B. cinerea* where optimum temperature for reproduction is 20–22°C at which eggs hatch in 4–5 days and the generation time is about 14 days. An undescribed *Aphelenchoides* sp. originally from rice stems obtained from Sierra Leone, is morphologically similar to *A. blastophthorus* and has an optimum temperature for reproduction of about 30°C, although it can reproduce at 35°C. At 30°C eggs hatch in three days and the generation time is about seven days. Males are common in both species and reproduction is bisexual. (Hooper and Pike)

Nematode galls on a moss. Galls on a moss, *Cirriphyllum cirrosum* sent by Mr. F. A. Sowter, Leicester, from a mountain forest in Rumania, contained eggs, juveniles and adults of either a *Tylenchus* sp. or a *Nothanguina* sp. The galls had a structure typical of that previously reported for other nematode induced galls on mosses. This is the first record of nematodes galling this particular moss and the first record of *Nothanguina* causing moss galls. Of the galls dissected, 27 contained *Nothanguina* sp., eight *Tylenchus* sp. and four both but in different parts of the gall. (Hooper)

Conjoint and other work

Cooperation was continued with Dr. C. E. Taylor, Scottish Horticultural Research Institute, on the identification of many specimens received for the NATO sponsored survey of the occurrence of *Longidorus* and *Xiphinema* spp. in U.K. and Eire. An unnamed species of *Longidorus* found by Mr. V. R. Mali, Czechoslovakia, associated with the roots of *Euonymus europaeus* L. is being described. (Hooper)

Staff

During the year the Department was in temporary quarters. In September D. L. Trudgill left to join the Scottish Horticultural Research Institute and in October C. D. Green went to the National Vegetable Research Station. P. Finch and S. Moss joined the Department in October. P. R. Mathews, Carol Shawcross, P. H. Pritchard and P. Rees

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worked as sandwich course students for six months. In February F. G. W. Jones attended the Tall Timbers Conference on Ecological and Animal Control by Habitat Management in Tallahassee, Florida, and visited centres of nematological research in Gainesville, Raleigh, Beltsville and Blacksburg, U.S.A. In July he was appointed Visiting Professor in the Department of Zoology and Applied Entomology, Imperial College, London. A. R. Stone attended a conference on potato cyst-nematode in Münster, West Germany, T. D. Williams attended a meeting organised by Eucarpia on breeding for resistance to cereal pathogens in Munkebjerg, Denmark and C. C. Doncaster visited the Institute for Plant Pathology and Plant Protection, Hanover, and the Institute for Scientific Films in Göttingen, West Germany. The Department participated in the 11th International Symposium of the European Society of Nematologists held in Reading in September during which 120 members visited Rothamsted.

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INSECTICIDES AND FUNGICIDES DEPARTMENT

I. J. GRAHAM-BRYCE

It is increasingly recognised that pesticides should be used more discerningly. Much of our work is directed towards achieving this by understanding the influence of environmental factors and formulation on pesticide behaviour. In practice only a very small fraction of the pesticide which is applied reaches the intended target, the remainder entering the environment without contributing to pest control. For example our work on persistence of insecticides on leaf surfaces and in ant baits shows that most of the toxicant rapidly evaporates into the atmosphere, particularly under tropical conditions, while our work with systemic compounds shows that less than 2% of the applied dose is likely to enter the plant after being applied to soil. There is therefore considerable scope for using pesticides more efficiently; large improvements should be possible by developing formualations and methods of application which relate the release of the toxicant and its pattern of distribution more accurately to the behaviour of the pest. The work on microcapsules and granular formulations illustrates this approach while also emphasising that the properties of the toxicant and the environment ultimately limit the extent to which pesticide behaviour can be controlled.

Favourable properties in the environment have no doubt contributed to the increasing use of the synthetic pyrethroids which we and others have developed. This group provides a range of different insecticidal and knockdown properties, but as a whole is characterised by very great toxicity to insects with extremely small toxicity to mammals and by short persistence. So far they have not found much use on field crops because they are relatively expensive and do not persist. However their rapid action, very great toxicity, the absence of residues and indications that resistance will be slow to develop give them considerable flexibility which may prove very valuable in future integrated control programmes, for example by enabling selectivity to be obtained by timing. The increasing interest in the pyrethroids should lead to new developments, widening the scope of the group still further. We are therefore maintaining our efforts to understand their mode of action and metabolism and the mechanisms whereby insects become resistant to them.

While conventional treatments with chemical pesticides must be the backbone of pest control for the foreseeable future, improvements coming mainly from making their use more efficient, our work on behaviour-controlling substances continues to suggest that more sophisticated methods may ultimately become practicable in suitable cases. At present the factors which determine how insects respond to most pheromones are not well understood and initially these compounds will probably find most use in environments such as food stores where conditions are reasonably predictable. The isolation and characterisation of the crowding pheromone from *A. kuehniella* and the demonstration that it occurs in other related stored-products pests is therefore particularly interesting. However, the broad potential of manipulating responses to behaviour controlling substances is shown by the observations that adding charcoal or extracts of oat plants to soil can decrease the ability of wheat bulb fly larvae to respond to exudates from wheat plants.

Work on the growth and health of crops in other departments at Rothamsted also frequently involves the use of pesticides and related chemicals. In the past, such studies

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