THE ACTION OF CERTAIN ORGANIC SILVER COMPOUNDS ON THE POTATO ROOT EELWORM, *HETERODERA SCHACHTII*, SCHMIDT,

with NOTES ON THE BIOLOGY OF THE PARASITE

and ADDITIONAL PAPERS

by

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The Action of Certain Organic Silver Compounds on the Potato Root Eelworm, Heterodera schachtii, Schmidt.

Of all the diseases to which the potato plant is subject, one of the most serious is that caused by the nematode worm Heterodera schachtii (Schmidt). This pest has now become widespread in Britain, particularly in the main potato growing areas such as districts of Ayrshire and Lincolnshire.

Historical

The existence of nematodes, or 'round' worms, has been known for several centuries and probably the first with which early naturalists became acquainted was the "vinegar eelworm". Goodey (38) notes that this organism was mentioned as early as 1656 by Petrus Borellus (4).

The first recorded information on plant pathological nematodes was given by Needham (70) who, in 1743, in the course of an examination of grains of smutted wheat, discovered galls caused by an eelworm now called Anguillulina tritici (Steinbuch) Gerv. & v.Ben. From this date our knowledge of nematode parasites of plants has been gradually built up. Although restricted at first by the limitations of the early microscope, it progressed by the investigation of galls and other obvious malformations on plants and the description of

the organisms contained in or giving rise to these abnormalities. Thus, in 1799, Steinbuch (96) examined galls which had been formed on the flowers of certain grasses, and recorded that they were caused by eelworms now known to belong to the genus Anguillulina. Naturally, greater interest was aroused and more attention given to the subject when the affected plants happened to be of agricultural or economic importance.

It was in England in 1855 that Berkeley (2) examined cucumber roots and found in them larvae of the root-knot eelworm known today as Heterodera marioni (Cornu) Goodey. Four years later, in 1859, Schacht (83), in Germany, discovered an endoparasitic eelworm in the roots of sugar beet. This worm was described more fully by Schmidt (84), who, in 1871, named it Heterodera schachtii. Further detailed investigations regarding its morphology, development and life history were carried out by Strubell (100), Chatin (13, 14, 16) and Liebscher (59, 60).

This nematode was shown to be the cause of a very serious disease of sugar beet in Germany, where it led to a condition known as "beet sickness" (rübenmüdigkeit), especially in fields which were given over to this crop without a suitable rotation. This condition was previously considered to be due to the exhaustion of the soil of certain essential minerals, particularly potash salts, but it was found that even heavy applications of fertilisers did not effect a cure.

The reduction in yield brought about by the pest was so great that in 1876 twenty-four sugar factories in Germany were closed down. It can therefore be understood that H. schachtii well merited its description by Baunacke (1) as "the dreaded parasite", and as "practically the most important nematode in Europe" by Marcinowski (61).

Beet sickness has been detected in the United States of America (88) as well as in European countries, and in Britain the first record of the disease was made by Petherbridge (76) in 1934, although, up to 1939, the beet eelworm has not been observed in Eire (9).

Other plants besides sugar beet were found to be attacked by H. schachtii. In Germany in 1874, Kühn (54) discovered the worm in the roots of oats, while those of barley and wheat were also found to be invaded. Damage to cereal crops, particularly oats, was also reported from Holland, Sweden and Denmark, and in 1930 from South Australia (17) (41). In Britain H. schachtii was recorded as a parasite of oats in 1908 and of wheat in 1909, but no severe infestation occurred until 1933 (Edwards (19)). In Germany in 1892, Liebscher (60) found peas and other leguminous plants attacked by the worm, which he concluded to be of a different species and to which he gave the name H. gottingiana. This is now considered to be synonymous with H. schachtii. Its occurrence as a parasite of peas in Britain was first noted in 1912 by Theobald (102) and reports of damage to peas from this cause were made in 1917 by Capus (6, 7)

in France and in 1931 by Triffitt (114) in England. Outbreaks of disease caused by H. schachtii have been recorded in this country also on tomatoes in 1936 by Johnson & Thompson (50), hops and other plants.

The occurrence of H. schachtii on potatoes was first noted in Germany by Kühn (55) in 1881, but this discovery appears to have been purely local since other authors, e.g. Hollrung (43) in 1891 and Vaňha & Stoklasa (117) in 1896, indicated that the potato plant was immune from eelworm infestation, and Kühn (56) himself in 1891 classed the Solanaceae as non-susceptible. Marcinowski (61) in 1909 recorded the uncertainty of the position but in her catalogue of host plants of H. schachtii she does mention Solanum tuberosum. She notes, however, that the list does not show the degree of susceptibility of the plants and says that some which become highly infested in some districts may be immune in others.

The potato plant was again shown to be attacked by H. schachtii by Hiltner (42) in 1909, but it was only in 1913 that Zimmermann (121), as evidence of the adaptability of the parasite, recorded its invasion of potato roots to such a degree as to make cultivation of the crop almost impossible in some districts of Germany.

Principally because the shape of the ripe female worm found on potato roots was more spherical than that found on sugar beet, the potato form was thought to be

a separate species. Thus in 1923 Wollenweber (119) named it H. rostochiensis and later Zimmermann (123) in 1927 called it H. schachtii, forma solani, while the synonym H. schachtii, subsp. rostochiensis (Woll.) was used by Kemner (52) in 1929. These names do not appear to have been universally accepted and the name Heterodera schachtii, given to the parasite in 1871, has been generally used for all forms of the worm. Lately, however, Franklin (28) has reopened the question and, considering the different races of the nematode to be separate species, she adopts for the potato strain the name suggested by Wollenweber, i.e. H. rostochiensis (Woll.).

In this paper, which deals with the attack of the nematode on the potato plant, the parasite will be referred to as the potato strain of Heterodera schachtii (Schmidt).

The disease of potatoes caused by this worm has been reported from Sweden and other European countries besides Germany. In Eire it was first recorded (8) in Rush, Co. Dublin in 1922, when it was associated with a disease noticed for ten years previous to this. The parasite has now become established in Eire, especially in sandy soil near the coast.

Occurrence of the Parasite in Britain

The potato plant is subject to attack by three eelworms - Anguillulina dipsaci (Kuhn) Gerv & v.Ben.,

Heterodera marioni and Heterodera schachtii. All are liable to set up a diseased condition of the plant, but although the first named may cause severe damage in clamps in certain localities, it is H. schachtii which is most widespread and the most serious menace to potatoes in this country. Moreover, amongst all the plants which this eelworm will parasitise under conditions prevailing in Britain, none is liable to such severe injury as the potato.

In 1909, Massee (62) stated that H. schachtii had been found attacking the roots of potatoes in Scotland. In 1916 it was recorded by Taylor (101) as occurring in two small gardens in Yorkshire, but not until 1923 was it discovered on field-grown crops there, and associated with a longstanding and obscure disease which had been first noticed in the district in 1904.

In 1919 severe outbreaks of a disease attributed to H. schachtii were reported in Cambridgeshire and Cumberland. Since then the parasite has become more widely distributed, until now almost all the potato-growing districts have centres of infestation. It has been recognised in Lincolnshire (67), Yorkshire (99), Lancashire and Cheshire (93) and many other counties. In Scotland, infested areas have been reported in Dumbartonshire, East Lothian and as far north as Ross-shire (73), as well as the early potato growing districts on the Ayrshire coast where H. schachtii

has become established as a major pest.

Symptoms of the Disease

Foliage: If the soil supporting the growth of potatoes has only a very slight eelworm infestation, the attack of the roots by the nematode may have no effect on the aerial parts of the plant. With the gradual accumulation of infestation in the soil, produced by consecutive crops of potatoes, definite signs of the disease do begin to appear. It may thus be several years before the presence of H. schachtii is detected and once discovered it may be present to such an extent as to have a serious effect on the crop. This is borne out by evidence given by Strachan & Taylor (99) from two fields in the same district in Yorkshire, which in a certain year produced fairly good crops of potatoes. The following year potatoes were again planted but in each case the crop was a total failure and H. schachtii was found to be very abundant in the fields.

The first evidence of eelworm invasion may be the slower rate of growth of the affected plants. This, however, may be readily overlooked if its real significance is unknown. The lowest leaves of badly infected plants have a tendency to wilt, wither quickly and drop off. In this way the lower part of the stem is left bare and the whole plant assumes a characteristic "feather duster" appearance, since the youngest leaves remain for a longer time apparently unaffected. These diseased plants are

easily recognised in the field as a result of their smaller size, and when several occur together in a badly affected area a typical feature is the "openness" of the drills, since the plants have not produced sufficient foliage to enable them to meet across the furrows.

Symptoms of eelworm disease in the individual potato leaf are first seen at the tip of the terminal leaflet, as a brown, often diamond-shaped patch, and this withered area gradually increases and, merging with similar necrotic areas which originate probably at the ends of the veins, extends over practically the whole leaflet. When this leaflet has withered the others fade in turn until the entire leaf is dead.

The withering of the leaves in this manner, which is often preceded by an inrolling of the leaflets reminiscent of the first stages of Leaf Roll, is probably due to the lack of actively absorbing rootlets by the plant owing to the penetration of these by the parasite.

Withering often takes place on one side of the midrib of the leaf before the other, so that while leaflets on one side are dead those opposite remain green and apparently healthy. O'Brien & Prentice (73) state that this characteristic is another diagnostic feature of potato eelworm disease, the foliage symptoms of which may in many cases be mistaken for those of potash deficiency.

Roots: Infection of the potato plant by H. schachtii takes place through the rootlets and although symptoms

caused by its effect on the underground parts of the plant may appear in the foliage, the activities of the parasite are confined solely to the true root system and it is never found in the stem or leaves.

In the roots as in the foliage, the onset of the disease is not apparent to the casual observer, for at this stage larvae of the worm make their way into the young rootlets of the plant and can only be seen with the aid of a microscope, when the roots have been suitably stained. H. schachtii, unlike the closely allied species H. marioni, causes no gall formation on the roots but, consequent upon invasion by larvae, slight swellings may occur in more strongly infected roots. With further growth of the roots these distortions disappear.

When an infected plant is uprooted at a later stage, small white seed-like bodies can be seen adhering to the surface of the roots. These are adult female worms in process of development and their colour gradually changes with maturity, first to yellow, then reddish brown, until finally they become mature dark brown resting cysts.

The larvae and immature cysts are generally found in the fine actively-growing rootlets and the more fully developed and mature cysts on the thicker root fibres. The reason for this is that the position of the female in the root is relatively constant, while the root itself, unless killed by the penetration of too many larvae, continues to grow after the attack, although

its vigour is restricted by the presence of the parasite.

In the comparatively early stages of a severe eelworm attack, the infected potato plant produces a large number of long, profusely-branched fibrous roots, and Marcinowski (61) notes the occurrence of these so-called "hunger roots" also on infected sugar beet plants. The finer branches of this mass of roots having been invaded by the parasite become functionless, dry and brittle to the touch, due to the destruction of the cortical layers by the eelworm, thus leaving the outer layers of each root fibre loosely attached to the woody vascular cylinder.

Much of the food material which the plant is able to absorb is used up in the production of new rootlets which are formed above the points of attack and thus nearer the surface of the soil. In many cases, therefore, the infected plants appear to have a well-developed root system. This conclusion is deceptive, however, since many of the fine rootlets are no sooner formed than they are killed or damaged by the invading larvae. As a result of this depletion of its absorptive system, the plant suffers greatly in periods of drought, thus indicating why damage done by the potato eelworm tends to be greater in dry seasons than in wet, since under the latter conditions the moisture encourages more root formation, which helps to counteract the effect of the penetration of the parasite. Further, dry conditions affect the shallower root mass of the infected plant more than the deeper

healthy root system.

The number of cysts formed on the roots is usually a measure of the severity of the eelworm attack, and O'Brien & Prentice (73) record finding as many as forty mature cysts on one inch of root, and as many as five young females in one transverse section of a rootlet. It is not uncommon to find over one thousand new cysts produced on one plant and, under ordinary field conditions, many of these cysts fall back into the soil when the crop is dug and provide fresh units of infestation for the next potato crop.

If infection is very severe, the plant may be so depleted of food material that it can produce no more rootlets and may die from lack of food and moisture. Generally, however, in common with other obligate parasites, H. schachtii does not cause the immediate death of the host plant.

The presence of cysts in any stage of maturity on potato roots is the infallibly diagnostic symptom of attack by H. schachtii. Conversely, however, it must be borne in mind that the absence of cysts does not indicate the absence of infection, since the plant may have been young and the cysts may not have had time to form or the infection may have been slight and all the cysts have fallen back into the soil. Cysts are not usually observed on the roots until at least one month from the time of the original invasion of the roots (64), and therefore, in the early stages of plant growth,

when the leaf symptoms suggest eelworm attack, the most accurate method of confirmation is by a microscopic examination of iodine stained rootlets (1) (31). An examination of the soil for the presence of cysts will serve to confirm the diagnosis.

Life History of the Parasite

Early investigations in the life history of H. schachtii were made by Strubell (100) and Chatin (13, 14, 16), and later some details of the life cycle of the potato strain were revealed by the researches of Goffart (35), Reinmuth (79), Triffitt (111, 112), Miles (64), O'Brien & Prentice (73) and others. A comparatively recent review of the life history and morphology was given by Goodey (37).

Potato eelworm infestation is carried in the soil through the agency of the resting cysts which contain variable numbers of eggs, sometimes as many as 700. These cysts are practically spherical in shape with a mean diameter of about 0.5 mm., and are furnished with a small neck at the anterior end. The eggs are cylindrical with rounded ends and measure about 0.1 mm. by 0.04 mm. The larvae which hatch from the eggs are variable in size, but average about 0.45 mm. in length and 0.02 mm. in breadth. The first-stage larva possesses a cuticle with transverse striations, a mouth with the mouth spear at the anterior end of the body, an intestine with which are associated clearly visible globules of reserve food

material, and a relatively pointed posterior end.

Experiments by Baunacke (1) and later by Rensch (80) with the sugar beet strain of H. schachtii revealed that the eggs of the nematode are stimulated to hatch by the presence in the soil of a substance produced by the roots of plants susceptible to attack by the worm. It is well known that many plants growing under normal conditions excrete soluble complex compounds from actively growing roots, and Triffitt (111) showed that the root excretion from the potato plant has the property of inducing the hatching of eggs of the potato strain of H. schachtii. This potato root excretion, whose composition is as yet unknown, was found to be non-volatile, to a certain extent thermo-stable, and to act only in presence of dissolved oxygen.

After the seed potato tubers have been planted and the young roots commence active growth, a concentration of root excretion is produced in the neighbourhood of the roots. This excretion affects the contents of potato eelworm cysts which lie dormant in the soil. Larvae hatch from the eggs and emerge from the cysts, make their way through the soil, and, apparently still influenced by the excretion, reach the fine rootlets of the plant. Then, by means of the mouth spear, the larvae penetrate the rootlet. These newly-hatched larvae are capable of travelling considerable distances through the soil. Baunacke (1) showed that larvae of

the sugar beet strain could penetrate through soil for distances up to 9 metres, and Fuchs (30) concluded that the range of their "wandering" was dependent on the soil temperature.

The larva enters the potato rootlet almost invariably just behind the root tip, in the region of cell elongation. Early investigators such as Baunacke (1) were of the opinion that entry of the root could only be made by way of some previous injury. Other workers, however, have shown conclusively that although an injury may provide a ready-made entry for the worm, invasion normally takes place through the wound caused by the mouth spear.

Having penetrated the rootlet, the larva then travels a short distance in the cortex towards the main root, rupturing the cortical cells in its course, and comes to rest parallel to the longitudinal axis of the rootlet with the mouth end pointing away from the root tip. In some cases where several larvae have penetrated the same rootlet, a larva may travel as much as 1 cm. into the parent root before coming to rest. Normally, further growth of the rootlet takes place and the larva comes to lie in the region of cell differentiation with the anterior end very close to the vascular cylinder.

The larva then commences a period of feeding and development and after about ten days a moult occurs, the larva shrinks from its old cuticle and emerges, still wormlike, as a second-stage larva, with a rounded posterior

end. After feeding for a further period of about ten days, the second-stage larva matures and becomes flask shaped with a sub-cylindrical posterior end, the gonad stretching about half the length of the body. From this point the development of the two sexes is different.

Male: The larva ceases to feed and the contents of the body shrink from the cuticle to which the spear and lining of the rectum remain attached, while a new cuticle is laid down. The rest of the organs become indistinguishable and the length of the body begins to increase. When the full length of the male worm (about 1 mm.) has been attained, the organs are complete and can be recognised. Thus, about 14 days after the second larval stage, the adult male is mature and lies folded on itself in the second larval cuticle. It eventually breaks out of the cuticle and makes its way through the cortex of the rootlet into the soil.

Female: The developing female continues to feed and becomes more swollen and flask-shaped. A new thick striated cuticle is laid down underneath that of the second-stage larva, but since no shrinkage of the body takes place, the two cuticles are difficult to distinguish from each other. The intestine increases in bulk and the genital tube, growing down towards the posterior end of the body where the rudimentary vulva has been laid down, unites with the vulva in such a way as to form a paired genital organ. Further growth and increase mainly in the intestine results in the shape of

the developing female becoming first broader and flatter, then club-shaped, and finally, when the organs are fully formed, spherical. According to Reinmuth (79) this stage is reached about 30 days after infection of the rootlets and thus the period of development of the adult female from the second-stage larva is about 10 days compared with 14 days in the case of the male.

As a result of the swelling of the female worm, the cortical tissues rupture so that the adult female, now ripe for fertilisation, hangs partially outside the root, with the mouth and head end firmly embedded in the root and the posterior end including the vulval region exposed. The young female at this stage is creamy white in colour and is believed to be fertilised by the male which may be found in the soil near the roots at this time.

After fertilisation, the ovarian tubes grow rapidly and absorb the food stored in the intestine. The whole body of the female, now a young cyst, continues to increase in size and, still attached by the head to the parent root, gradually becomes darker in colour until it is dark brown at maturity, which occurs about 30 - 35 days after fertilisation, i.e. about 70 - 80 days after the planting of the potato. These figures are by no means absolute, since variations in the temperature of the soil appear to have an effect on the duration of the different stages of the life cycle (73).

During this time the egg cells in the cysts develop

and take on a chitinous covering beneath which the larvae are formed.

Early investigators, such as Chatin (16) and Fuchs (30), were of the opinion that several generations of the beet strain of H. schachtii were passed in one year, and that the majority of the cystic females produced in summer remained white in colour and liberated larvae immediately to infect fresh roots. They concluded that brown cyst formation increased in autumn and served to protect the nematode over winter. Later German workers still contend that the beet strain can produce several generations in one year, e.g. Schmidt (85) states that as many as nine may be passed in one year.

Sengbusch (87) and Triffitt (112), working with the beet and potato strains respectively, showed that all the white immature cysts could change to brown cysts. Triffitt also determined that larvae are only liberated from the brown cysts of the potato strain and even then only about one month after the removal of the cysts from the potato root. She thus came to the conclusion that only one generation of the worm was passed per year and this finding was confirmed by O'Brien & Prentice (73) using both early and late varieties of potatoes.

In this way, brown resting cysts are formed, which serve to protect the eggs and embryonic larvae. When these cysts fall from the roots of the plant they remain dormant in the soil to provide a source of infection for a further crop of potatoes.

Effect on the Plant

Internal Effect: The vascular elements of either rootlet or root of the host plant are never invaded by H. schachtii larvae but a disturbance in the stelar tissues in indirectly brought about. When the larvae lying longitudinally in the cortex commence a feeding period their heads abut on the endodermis. Their presence in this position results in the formation by the plant of enlarged parenchyma cells immediately outside the endodermis close to the head of the worm. To these cells Nemec (71), who recorded their occurence in infected sugar beet, gave the name "giant cells". Triffitt (113) and O'Brien & Prentice (73) recorded minor differences in giant cells formed in the potato plant compared with those produced in the sugar beet but their function appears to be the same in each case.

By the breakdown chiefly of their transverse walls, the giant cells form large, multinucleate syncytia which are rich in granular protoplasm. These cells, by inward growth towards the centre of the root, displace the elements of the vascular cylinder, particularly those of the xylem, the continuity of whose larger vessels is often broken, while cambium development is arrested and growth of metaxylem and metaphloem ceases. Thus a serious interruption is caused in the conduction of water and food material through the stele of the infected roots to the aerial parts of the plant and also in the translocation of sugars and other substances from the

leaves to the growing rootlets. New rootlets are formed but these also are quickly attacked and rendered functionless. In this way it can be seen why a badly infected plant wilts quickly in bright sunshine, and why food reserves cannot be stored in any large amount in the tubers.

The giant cells appear to be concerned with the nutrition of the parasite and may from their richly granular contents provide it with an abundant food supply which it absorbs apparently by means of the hollow mouth spear. The formation of giant cells, in the opinion of Nemec (71), is caused by the excretion of some toxic substance by the larva. This substance, as well as the products of excretion of the parasite, may ultimately be transported by the vascular system to the leaves where it may accumulate in the regions of rapid transpiration, i.e. near the hydathodes round the margins and at the apex of the potato leaflet. These are the positions, according to O'Brien & Prentice (73), where obvious symptoms of the disease are first evident, i.e. where withering commences.

Hellriegel (40) showed that the sugar content of sugar beet heavily parasitised by H. schachtii was much lower than that of healthy plants. A deficiency of potash has also been found in infected plants and at one time the lack of this element was considered to be the cause of the disease, the more so when a beneficial effect was obtained by the application of potash to the infested soil.

The presence of potash in the plant tissues may, however, serve to neutralise the toxic substances secreted by the nematode, as was suggested by Steiner (97), who concluded that the damage to the plant rather than being mechanical is produced by the toxins.

It was also shown by Stoklasa (98) that the leaves of sugar beet plants whose roots were severly infected by H. schachtii contained only about half of the normal quantity of lime but possessed a large amount of oxalic acid. Smith (95) has shown that the formation of giant cells in plants is, as in the production of tumours, stimulated by the liberation of weak acids or alkalis by the parasite. The secretion of oxalic acid by the larvae in the case of sugar beet and possibly also in potatoes may thus not only stimulate the formation of giant cells but also produce the withering symptoms in the leaves.

Effect on the Crop: The ultimate economic effect of attack by H. schachtii on the potato plant is to reduce the size of the crop of tubers. The method whereby this is brought about has already been indicated. The loss of crop due to potato eelworm in the early districts of Ayrshire is on the average about 30% but in some years has been estimated to be as high as 70%. The financial loss entailed is substantial, when one considers that in the Ayrshire region, in normal times, some 4,000 acres are given over annually to the early potato crop and that the average value is about £50 per acre.

If the weather during the growth of the plants is very dry, the effect on eelworm infected plants is usually severe owing to the lack of absorptive rootlets, and if prolonged drought occurs the crop may become a total failure. If in the early stages of growth conditions are warm enough to allow the plants to become established and yet not sufficiently warm for the larvae to hatch in large numbers, the damage caused by later invasion of the roots is not so great. The minimum soil temperature at which larvae emerge is about 6° C. and the optimum about 18 – 20° C.

The yield from infected plants does not appear to be decreased to the same degree with early crops as with late ones according to O'Brien & Prentice (73), who give the reason for this as the higher soil temperatures obtaining during the growth period of late crops, which are more favourable for larval emergence immediately from the time of planting of the potatoes. The average yield of early varieties on badly infested soil, as given by these workers, is 2 - 3 tons per acre and for late varieties 15 - 20 cwt. per acre. These figures, however, cannot be directly compared because of the differences in cropping potentialities of different varieties and varying amounts of infection in different fields, but they may indicate, from an agricultural standpoint, the general tendency for the effect of eelworm attack, at any rate in Ayrshire, to be more serious on late crops than on early crops. On the other hand, Morgan (67)

in Lincolnshire and Zimmermann (122) in Germany found greater decreases in crop in early varieties. It may be a question of differing soil temperatures or even different methods of cropping.

Since H. schachtii attacks only the true root system, the tubers from infected plants remain quite sound, although tubers have been found with large numbers of cysts adhering to the skin.

Secondary Infection

Where continuous cropping has occurred for a number of years the soil becomes very heavily infested with eelworm cysts and acquires a condition known as "potato sickness". This is often particularly severe in patches, where only a few of the plants attain normal size and many of the others die off prematurely. Baunacke (1) observed a similar phenomenon occurring in "beet sick" soil and explained it as being due to heavy eelworm infection resulting from an abnormally large cyst population in these areas, which are known as "nematode nests".

Attempts to correlate potato sickness with cyst counts of the infested soil have in the majority of cases failed. Morgan (68) and others have found as many cysts in soil with a satisfactory crop of potatoes as in areas of severe damage. Further, plants which had large numbers of female worms attached to the roots were observed to be in an otherwise apparently healthy condition and gave a relatively normal crop of tubers. Smith & Prentice (93)

found that where the infestation had been recently introduced, the apparent amount of disease corresponded with the cyst counts. After 3 - 4 years, however, no correlation between these two factors could be established.

In view of these facts, it was suggested that H. schachtii was not the sole cause of the damage and that there was possibly some other factor associated with the nematode. The effect of this secondary factor was concluded to be most evident where continuous cropping had been practised. Morgan (68) suggested that it might be connected with soil fertility or with the attack of a fungus, e.g. Rhizoctonia (Corticium) solani (Kühn), present in many eelworm infested soils, but Buckhurst & Fryer (5) showed that this fungus was definitely not associated with H. schachtii in causing potato sickness.

It was found by Triffitt (113) that when H. schachtii was the only pathogenic organism to attack the potato, the early check to growth might be made good by an increase in the production of secondary roots. The secondary factor was thought to be temporarily eliminated by partial sterilisation by steam or by certain chemicals as in experiments by Buckhurst & Fryer (5), Roebuck (82) and Edwards (18), or by the adoption of a suitable rotation combined with judicious manuring as suggested by Morgan (68), who concluded that "the problem was primarily one of obtaining more suitable conditions for plant growth". Buckhurst & Fryer (5) found no evidence of potato sickness in soil which had been steam sterilised

and to which cysts had been added, but severe symptoms were seen on plants growing in unsterilised soil with the same cyst content, and they associated the secondary factor with a slow initial growth which is not made good by ordinary manurial treatments. It was thus considered that the unknown factor set up a soil condition unfavourable for the production of rootlets necessary to overcome the check caused by eelworm attack.

In the light of later work, such as soil sterilisation experiments by Carroll & McMahon, many of these facts may be explained without recourse to a secondary factor. These workers (8, 10, 11), as well as confirming the findings of Buckhurst & Fryer, showed that sterilisation of infested soil had a temporary inactivating effect on the root excretion of the plant resulting in temporary stoppage of larval emergence. It was also shown that when cysts were removed from infested soil by seiving, plants grown in the soil did not show signs of potato sickness which, however, did appear when the cysts were replaced in either the extracted soil or in clean soil. They further showed that when larvae were introduced into newly sterilised soil in which potatoes had been planted, severe potato sickness was caused.

O'Brien & Prentice (73) pointed out that in Ayrshire the areas of severe infection occurred in very sandy parts of the fields and they connected the extreme damage to the plant with (a), early stimulation of the nematode caused by early heating of the soil and (b), drought

conditions in sandy soil adversely affecting the already inefficient root system.

It has already been noted that Smith & Prentice (93) could find no correlation between the amount of disease and the cyst content of soil carrying the disease for over three years, and it often happens that fields giving a high cyst count of e.g. 10 per gm. may produce a smaller infection than those with 2 cysts per gm. The infection depends primarily on the numbers of larvae which enter the roots and in soil samples with many cysts, only a few of these may be capable of liberating larvae at all and the productivity of even these may be small.

The damage done to the crop also depends on the time of penetration of the larvae, since the host plant can withstand a much heavier infection if the attack takes place after the plant has been well established. The potato suffers most when the larvae are able to invade the first formed roots. This was neatly demonstrated in an experiment described by Leiper (58). By interposing a layer of clean soil round the tuber, eelworm attack was delayed and the plant produced, although obviously having suffered a setback, was much superior to that grown wholly in infested soil and thus subject to immediate attack.

When the eelworm larva enters the potato rootlet and subsequently makes its way through the cortex, and when the adult male and female worms break out of the

root, the mechanical injuries caused provide easy access for any parasitic bacteria or fungi present in the soil. This is true secondary infection. Fungi were found in dead syncytia in sugar beet by Nemec (71) and Triffitt (113) has observed the fungus Colletotrichum atramentarium in the giant cells and xylem parenchyma of potato roots invaded by H. schachtii larvae, while unattacked roots did not harbour the fungus.

This secondary or fungal infection may accentuate the damage done by the nematode which is the principle cause of the disease and which acts as a true parasite by drawing all its nutriment from, and undergoing its development in, the host plant.

Soil Conditions

Although potatoes may be grown almost anywhere in Britain, only certain areas whose soil has proved capable of producing heavy or early crops are given over to intensive cultivation of the plant. The loose texture and good aeration of light and sandy soils has been found most suitable for potato growing, especially of early crops. It is in this type of soil, however, that the parasite also appears to thrive best and thus large areas in Ayrshire, Lancashire, Yorkshire and Lincolnshire are not only centres of potato cultivation but have also become centres of eelworm disease and foci of infestation.

That potatoes suffer more from eelworm attack when

growing in light soils than when growing in heavy soils was demonstrated by both Reinmuth (79) and Triffitt (111) who also observed the tendency for cysts produced in the heavier soils to be smaller than those from light soils.

Another factor which plays an important part in eelworm infection, especially in light soils, is temperature, and early warming of this type of soil in spring not only causes earlier and quicker growth of the plants but also brings about an early invasion of the first formed roots.

The soil reaction appears to bear no definite relationship to the severity of the disease, although obviously plants grown in soils either too acid or too alkaline would tend to suffer more from an equivalent eelworm attack than plants in soils of suitable acidity. For some time it was believed that the acidity of a soil and its cyst content could be correlated, but further experiments disproved this for light soils, although Smith (92) claims that a negative correlation exists between these two factors in peaty soils.

Cysts may persist in the soil for a number of years. They do not liberate all their larvae in the first year, but continue to set them free over a period of years. Through time, however, in a field in which potatoes are annually grown, the older cysts become empty and thus in a sample from such a soil, cysts in all degrees

of fullness will be present. Although some of the eggs in these resting cysts may remain viable for seven or eight years in the absence of the host plant, the infective potentiality of the cyst population as a whole decreases from year to year (Franklin (24)). Even neglecting the unequal distribution of the cysts in the field, it may thus be seen how unreliable cyst counts of infested soil may be as an invariable criterion indicative of the amount of eelworm infection.

The cysts are most numerous in the top nine inches of soil, not many being present below this level although they have been found at a depth of two feet. Baunacke (1) in his work on the beet eelworm found cysts as deep in the soil as 80 cm. ($31\frac{3}{4}$ inches) and suggested that the larvae from those in the cultivated layers effect an immediate attack on the host plant, while the cysts deeper in the soil are the true carriers of a permanent infection.

Franklin (23) found that H. schachtii larvae will penetrate potato roots after remaining in the soil from autumn until the following summer. Larvae which are hatched late in the season and cannot find a host plant to invade may thus over-winter in the soil and be ready for an immediate attack when the new tubers are planted.

It may be concluded that where no rotation of crops is practised eelworm infestation in the soil may be increased

to a very high degree by the building up of a large population of viable cysts. If this process went on indefinitely it would be impossible to grow potatoes at all in the affected areas. As is the case with most true parasites, however, a natural balance is set up. Where the ground is so heavily infested that the plants are overcome by the attack, a great reduction in the amount of soil infestation may result, since the death of the plant in which the nematode is still developing causes also the death of the worm. Thus the disease is self-limiting and this fact explains why potatoes may sometimes be grown on a field in which the crop has completely failed in the previous year. "Trap cropping", a method of attempted eelworm control to which reference will be made later, is based on the same principle.

Spread of the Disease.

The distances which freshly-hatched larvae are capable of travelling have already been noted and the spread of the disease ultimately depends on the larvae reaching a host plant. Once a centre of infestation has been introduced into the soil, the disease spreads and the infested patch increases but slowly, if external agencies do not intervene. The distance of 8-10 feet has been suggested for the annual rate of spread (73).

Actually, the normal farm practices of ploughing, harrowing, etc., play an unavoidable part in mixing the cyst-containing soil and spreading the infection to other parts of the field. The cysts are light and can withstand drying and when in this condition they readily float on water. In this way they may be spread by rain or drainage water, or when dry and lying on the soil surface they may be wind-blown to another part of the field. If the cysts newly-formed on the roots do not immediately drop off, they are usually shaken off in the process of digging the crop and return to the soil to increase the infestation in the field.

The disease is also spread from field to field through the agency of cysts contained in earth which adheres to farm implements, to the hoofs of horses and to the boots of the farm workers.

The parasite is not spread by the droppings of animals which have happened to feed on any part of an infested field. This was shown to be the case with the beet strain of H. schachtii by Chatin (15), who fed samples of diseased beet to sheep, and by Triffitt (108) who found that the contents of potato eelworm cysts had been killed by being passed through the alimentary tract of young pigs.

The most important means of spread is that whereby clean land in an uninfested district becomes contaminated,

and in almost all cases this takes place through cyst-bearing soil adhering to seed potatoes. It is possible also for the roots of sprouted tubers to become infected through these adhering cysts before ever being planted. The method of marketing "ware" potatoes provides a common source of spreading the disease (73) (58). When potatoes grown on infested land have been "barrelled" some of the adhering soil, which probably contains cysts, is rubbed off and collects at the bottom of the barrel. Also the barrels are usually topped with potato haulms with roots attached on which there be as many as 1000 cysts. Once the potatoes have been sold, the barrel may be sent to another grower whose land is uninfested and here the contents of the barrel may be cleaned out and so provide a new centre of infestation.

The spread of the disease through cysts adhering to seed potatoes may be prevented to some extent, according to Franklin (25, 27), by treatment of the tubers with 5% formaldehyde for 5 - 6 hours or with 1% formaldehyde at 185°F. for 20 minutes. Some varieties of potatoes, however, appear to sustain damage as a result of such treatment.

Adaptability of H. schachtii.

It is considered probable that H. schachtii was originally polyphagous having as hosts a wide range of weeds

and cultivated plants, and in the opinion of Baunacke (1) its effect on these plants was relatively slight. When a suitable host plant is grown in close rotation or with no rotation in soil where the nematode is present, the latter appears to adapt itself to the crop which it attacks strongly and there follows the development of a specialised strain or biologic race of the parasite which is monophagous or which will attack only a very limited range of plants. This monophagous strain displays greater powers of multiplication and spreading than polyphagous races, according to Baunacke (1), who describes the sugar beet strain. Moreover, the attack by a specialised strain is much more intense, and the damage suffered by the plant much more severe, than by an unspecialised race.

While certain unspecialised strains may be present in this country, Goodey (37) notes that there is evidence of the occurrence of at least four distinct biologic races each with a fairly limited host range. (a) The beet race, with a host range among the Chenopodiaceae and Cruciferae. (b) The potato race which has also been occasionally found on tomato and other Solanaceae and rarely on certain of the Chenopodiaceae, Graminaceae and Umbelliferae. (c) The pea race which also parasitises beans and other legumes. (d) The oat race which attacks cereals and certain grasses and has also been found on red clover.

Much discussion has taken place regarding the identity of these strains, especially the relationship between the oat and the beet races, and the occurrence of "major" and "minor" larvae in the former (36). The main morphological characteristic which distinguishes the potato strain from the others is the rounded shape of the cystic female which in the other races is lemon-shaped. Certain further differences between the strains have recently been shown by Franklin (28) who advocates that the four races should be regarded as separate species.

Early German investigators considered that the potato was not readily attacked by H. schachtii, indeed Vaňha and Stoklasa (117) stated in 1896 that it was the "very plant which the genus Heterodera avoids". Thus arises the interesting question of the original source of potato eelworm disease. Baunacke (1) suggested that the beet strain would also parasitise the weeds of the beet field, one of the chief of which is Solanum nigrum, L. (black nightshade). This plant becomes highly infected by H. schachtii and it is not unnatural that the closely related S. tuberosum, L. (potato) is also soon attacked and used as a host plant. It is possible, however, that the parasite does not need an intermediate host and can attack directly plants of another family. On the other hand, it may have been a polyphagous race which originally attacked

the potato so that this strain need not have evolved through the beet race. Although there is no direct evidence, the probability is, according to Tiffitt (116), that natural infections on weeds and certain grasses were the original sources of infection on agricultural crops.

Natural infections of H. schachtii have been found in this country on several plants, including Ammophila arundinaceae, Host, (marram grass) by Triffitt (110). Two distinct strains of H. schachtii have also been observed in this country by Franklin (26) to occur naturally on two common pasture plants, Agrostis sp. and Trifolium sp.. In the U.S.A., beet sickness is prevalent in certain districts of Utah, and in that State the nematode has also been discovered (104) parasitising the roots of a weed of the same family as beet (Chenopodiaceae). This infected weed is common in areas where beet has never been grown and it is very probable, according to Goodey (38), that infections of sugar beet by H. schachtii in Utah have originated by transference of the parasite from the weed to the crop.

The potato strain of the nematode is very highly specialised to a limited host range and consequently it will not adapt itself readily to other plants. Goffart (35) succeeded with difficulty in effecting its transference to sugar beet, but the cysts produced were lemon-shaped like the

beet, oat and pea strains. Both Morgan (67) and Reinmuth (79) have found tomato roots to be attacked by the potato race. The former worker has also recorded (67) a light infection of Chenopodium album, L., by this strain, and its attacks on Agropyron repens, Beauv., (couch grass) and carrots were observed by Triffitt (110, 116).

As a general rule, the highly specialised potato race of the nematode although causing severe damage to potatoes does not attack the weeds of the potato field. In the absence of its specific host, however, it is possible that this strain may invade the roots of the common field weeds, as has been reported by Triffitt (109) for another strain which attacks mangolds. If this were to happen regularly the parasite could maintain itself on a wide range of naturally occurring plants, and thus conserve a permanent source of potential infection.

Control of the Parasite.

Since the discovery of the pathological significance of the eelworm H. schachtii, many methods have been elaborated in attempts to control the parasite. The problem has been approached from several angles, but whatever the crop attacked adequate remedies have been difficult to devise since the infection is derived from the cysts of the parasite which are

distributed throughout the soil. The two main avenues of investigation, having as their end the control of the nematode, may be broadly described as (A) biological and (B) chemical.

(A) Biological Control.

(1) Inoculation by fungi. Certain fungi are known to be able to attack parasitic nematodes including H. schachtii. Goodey (39) records that the stem eelworm Auguillulina dipsaci, which was found invading the leaf stalk of Calceolaria integrefolia, Murr., was itself attacked by an eelworm-trapping fungus Arthrobotrys oligospora, Fres., although how the fungus penetrated the plant was not known.

The cysts of H. schachtii themselves harbour several fungi including, according to Baunacke (1) Isaria destructor and Tarichium auxiliare, and in certain cases the eggs and developing larvae in the cysts have been found to have been destroyed by fungi. Goodey (37) states that Korab (53) found cysts of the sugar beet race to be heavily parasitised, under natural conditions in South Russia, by fungi chief among which was Torula heteroderae which causes the eggs and larvae to turn reddish brown in colour. Some of these cysts also contained the fungus Arthrobotrys oligospora.

Methods to control H. schachtii by means of fungal cultures have been devised, but Marcinowski (61) records that

they yielded doubtful results and cost so much that little prospect was held for their practical application.

(2) Rotation. The potato strain of H. schachtii causes the most severe damage where potatoes are cultivated annually on the same land. If rotation of crops is adopted the risk of serious loss is greatly diminished, and it is recognised that although the contents of the cysts may be viable after remaining in the soil for even 8 years in the absence of the host plant, satisfactory crops may be obtained by withholding potatoes from badly infested soil for 5 or more years. Zimmermann (122) noted the benefit to subsequent crops of avoiding growing of potatoes for 3 years, and O'Brien & Prentice (73) stated that absence of potatoes for only 1 year had a beneficial effect in some fields in Ayrshire. The value of crop rotation in connection with potato eelworm disease was also stressed by Morgan (68).

If a rotation is adopted, it is advisable to keep down weeds, which could possibly become infected and thus serve to propagate the parasite, although this does not appear likely with the potato strain of the nematode which is considered to be highly adapted to its host plant. Consequently most of the common agricultural crops may be grown without risk of infection on potato sick land provided the soil is suitable for their cultivation.

The practice of a fairly wide rotation is still the most effective procedure which the grower can adopt if a satisfactory crop is to be obtained from badly infested land. This method is often not economically practicable, as in the case of light sandy soils which are most suited to the growing of early potatoes, and an extreme example may be seen in the case of one field on the Ayrshire coast near Girvan, in which early potatoes have been grown annually with no rotation for almost 100 years. Such soils are unsuited to other crops and hence there arises the difficulty of finding a remunerative substitute for the potato.

(3) The Use of Plants to inhibit or stimulate larval emergence. In 1925, Morgan (67) showed that when mustard was grown along with potatoes in soil infested with H. schachtii, fewer cysts were formed on the roots of the potatoes than when this crop was grown alone. Triffitt (107,111) confirmed this and found that when mustard was turned into the soil there was a definite inhibition of cyst development on the roots of potatoes grown in the soil. This was probably due to the neutralisation or masking of the potato root excretion by that of the mustard. Further use of mustard in field trials, however, was not so successful.

Triffitt (115) also showed that the root excretions of certain grasses especially Poa trivialis, L., and P.

pratensis, L., rough-stalked and smooth-stalked meadow grass, stimulated larval emergence from the cysts and it was found that these larvae did not penetrate the roots of the grasses. In a small-scale field experiment in which eelworm infested soil was sown with a grass mixture containing a considerable proportion of the two grasses already mentioned, a reduction of 33% of the viable contents of the cysts in the soil was obtained in 12 months and 48% after 18 months.

Maize has been found by Franklin (22) to have an effect similar to that of the meadow grasses but not to such a marked degree.

The larvae liberated by these methods do not penetrate the plant roots and may die off or be killed by the application of suitable chemicals.

In order to attack the contents of cysts deep in the soil, Baunacke (1) proposed the sowing of "activation" plants during the period of maximum development of the nematode and suggested that the adoption of this method in conjunction with a rotation and the use of "ammonia" would, in a few years, empty the cysts.

(4) Trap cropping. This method of combating the nematode was devised by Kühn (56) in 1891 in his investigations on the sugar beet strain, and has been tested extensively in Germany. The principle of the method is to sow a quick-growing

susceptible crop in the infested field and to have it uprooted and destroyed after it has been invaded by the larvae and before the female worms have had time to develop to sexual maturity.

If the strain of the nematode is highly specialised, no other suitable crop can be sown in place of the host plant. In this case the host itself may be used and this has been done, with encouraging results, by Carroll and McMahon (11, 12) in experiments on the control of the potato strain. They showed that the required decrease in infection was not effected in potatoes planted immediately after the trap crop had been lifted, because of stimulation produced in the cyst contents by the trap crop, which results in the early infection of the main crop. It was thus necessary to plant a second trap crop or allow the land to remain fallow, either of which procedures entailed the postponement of the planting of the main crop until the following season. From these trials it appears that if the trap crop is planted in April, it should be removed after 5 weeks, and if planted in May, removal should take place after 4 weeks, owing to the more rapid development of H. schachtii in warmer conditions. If two trap crops are employed, the first should be planted in early April, removed after 5 weeks, the second planted afterwards and lifted 3 weeks later.

The trap crop may be successful even if roots are

left in the soil, although some of the worms can develop and produce small cysts even if the roots have been severed. Rhizomes of the plant should not be allowed to remain in the soil since they may produce new buds and eventually new plants with more cysts. It was found more satisfactory to plant the tubers fairly near the soil surface and to dig the trap crop, rather than lift it by hand. If the trap crop is allowed to remain in the soil longer than 50 days after larval invasion, the residual soil infestation is no less than that produced by plants left until maturity.

The adoption of this method of control, successful though it may be, means that potatoes cannot be grown as a crop in the field for at least one year, and although Carroll & McMahon suggest the practicable possibility of growing the trap crop between the drills of another crop, such as beet or turnips, and removing it before it began to compete seriously with the latter, yet the method has up till now not been generally practised.

(5) Resistant or Immune Varieties. Almost complete resistance to attack by the oat race of H. schachtii has been found in certain varieties of barley by Nilsson-Ehle (72), and these may be used with advantage in rotations of cereals in soils where the oat strain of the nematode occurs. Baunacke (1) mentions that the breeding of a resistant variety of sugar beet

was advocated by Wilfarth and that selection experiments were conducted by Muller & Molz, but apparently without much success.

No variety of potato has yet been evolved which is immune from the root eelworm, but it has been shown by Robertson (81) that some commercial varieties are apparently more susceptible than others. Of all the varieties tested in heavily infested soil in Aberdeenshire over a period of eight years, Epicure was the variety which consistently produced the heaviest crop. Although Epicure, grown on clean land, usually gives a higher yield than the other varieties in the trial, from a comparison of the relative weights of the crops, Robertson concluded that Epicure exhibited a comparatively high resistance to the effects of eelworm invasion. There was no question of this or any other variety resisting the attack of the larvae, since all showed an equally large number of cysts on the roots. Hence it is possible that this strain of Epicure was relatively resistant to the effects of eelworm disease, which according to Steiner (97) are toxic rather than mechanical.

It is another matter and a more difficult task to produce a variety of potato which will not only be resistant to eelworm invasion, but also make an appeal to growers because of its satisfactory cropping powers and to the public by reason of its size and quality.

(B) Chemical Control.

The use of chemicals for the control of plant diseases dates back to the times of the ancient Greeks and Romans. Cato, in 200 B.C., recommended the fumigation of trees with sulphurous smoke to eliminate the "vine fritter". Probably the first observations of a truly scientific character on the effect of chemicals on plant diseases were made in 1807 by Prévost (77) who investigated the action of copper sulphate on the spores of wheat smut.

It would naturally be supposed that, in the absence of an immune variety of host plant, the ideal method of eelworm control would be the application to the soil of small quantities of a cheap chemical which would have a rapid lethal effect on the cyst contents, without having a harmful action on the plant. This compound ought not to be difficult to apply and should produce no injurious effect on the soil, but rather be of manurial value.

With a substance in view having all or some of these attributes, much work has been done especially with respect to the beet and potato strains of H. schachtii. The application of certain compounds may have the effect of improving the yield of potatoes, but yet have no toxic action on the parasite itself. Such substances cannot be classed as nematocides and such treatment is not a control measure but merely an economic

expedient.

Many substances have been tested and many methods have been devised principally along the following lines:- (1) to induce larval emergence in the absence of the host plant and to destroy the larvae, (2) to neutralise or inhibit the action of the host root excretion, (3) to inhibit the hatching of cyst contents and (4) to destroy the cyst contents and the free larvae.

(1) To induce larvae emergence. Rensch (80), working on the sugar beet strain of the parasite, made up a solution chemically similar to the excretion of sugar beet roots, and applied it to "beet sick" soil where no crop was growing. He hoped that by this means the soil would become so impregnated with the solution, that all the cysts would be induced to liberate their larvae which would die in the absence of a host plant or be killed in the unfavourable conditions during winter. Moreover in spring when another host plant was sown, the soil was again impregnated with a solution chemically similar to the root excretion of the plant, so that the newly-hatched larvae would not receive a direct stimulus from the plant, owing to uniform dispersal of the activating solution in the soil. This method was, however, unsuccessful under field conditions, partly on account of the cost.

Following up this line of attack, Nebel (69) and

Rademacher (78) used coal tar and animal oils which, at certain concentrations, were reported to stimulate hatching and later to kill the larvae.

Molz (65) found that dilute solutions of bleaching powder, especially if used in conjunction with quicklime, increased the emergence of the larvae of the beet strain which were then killed by the action of these substances. It was suggested that non-susceptible crops should be grown for two or three years after this procedure has been carried out in the field, until all the larvae were dead. Although this mixture of compounds was cheaper, more soluble and more rapid in action than animal oil, its effect was apparently weaker and was not maintained for so long.

Applying the same principle to the control of the potato strain of the nematode, Reinmuth (79) devised a method whereby a decrease in the cyst content of the soil and increase in crop was claimed. As soon as the potatoes were lifted the haulms were cut into small pieces and turned into the soil. A catch crop of either lupins or sweet clover was then planted, ploughed in early in autumn and stinking animal oil, absorbed in sawdust, applied as a top dressing at the rate of 6 cwts. per acre. The purpose of the catch crop remains obscure, but the animal oil was said to stimulate hatching of the cyst contents and then kill the larvae. No stimulation was, however,

observed by O'Brien & Prentice (73) using this substance in laboratory tests.

One of the most effective chemicals in producing stimulation of hatching has been found to be bleaching powder. Smedley (89), using cysts of the potato strain, showed that low concentrations of certain halogen compounds, including bleaching powder, have this property to a very marked degree. In stronger solutions these substances can dissolve the cysts. It was noted that hypochlorite solutions containing calcium can attack the egg membrane of H. schachtii, so that the larvae may hatch without the presence of natural stimulants such as the root excretion of the potato plant. Unfortunately, however, hypochlorites cannot be used on a commercial scale, because of their instability in the soil.

(2) Neutralisation or inhibition of the action of root excretion. If the exact chemical composition of root excretion were ascertained, greater advances would be possible along this line towards the control of the parasite which it stimulates. However, certain information is known about its reactions, among which is the fact that potato root excretion from soil which has been recently sterilised does not possess the same power of hatching as does that produced in unsterilised soil. This was shown by Carroll & McMahon (10, 11) who found that when cysts are added to sterilised soil in which potatoes have

been planted, larval emergence takes place only after an abnormally long period. In the case of recently sterilised soil the time lag is greatest, being about 30 days, but the effect gradually diminishes through time, and is negligible in soil which has been sterilised for three months. Soil sterilisation produces a temporary effect on the potato root excretion and not on the contents of the eelworm cysts. Its action, therefore, delays the attack by the nematode until the plant is established and thus better able to overcome the effects of larval invasion.

Thus Buckhurst & Fryer (5) noted temporary beneficial results by partial steam sterilisation of the soil, and Roebuck (82) and Edwards (18) obtained increased yields after applications of naphthalene since it is probable, according to Goodey (37), that this compound was acting as a partial soil steriliser and not as a vermicide. Morgan (67), Miles (64) and Buckhurst & Fryer (5) also reported an improvement in the potato crop after treatment of the soil with naphthalene, but observed no diminution of the cyst content of the soil. Mercuric chloride may also have a sterilising effect on the soil, since Johnson (49) found that, although an increase in crop was produced and the degree of infection of the plants reduced after application of this compound, it did not kill the cyst contents, but merely delayed larval invasion of the roots.

The application of a method of incomplete soil sterilisation to the problem of eelworm control must therefore be considered only as a temporary expedient, since it accomplishes little in the eradication of the pest, and cysts are produced in the usual way although their formation is delayed.

(3) Inhibition of hatching of cyst contents. If larval emergence is to be inhibited, the cyst contents must be inactivated or kept separated from the young potato roots. The result is similar to that of the previous line of attack, in that a delay is caused in the penetration by the larvae into the roots of the host plant. The length of this delay will determine the effectiveness of the method, for if the retardation is long enough, the worms which eventually attack the plant will be unable to complete their life cycle and no new cysts will be formed.

This may be done by applying certain chemicals, e.g. Calcium chloroacetate, at rates which are not lethal to the cyst contents. The actions of some of these substances will be discussed in the next section. The application of heavy dressings - 20 tons per acre - of farmyard manure to the drills, enabled potato plants to withstand the attack of H. schachtii and gave very satisfactory results according to O'Brien & Prentice (73). Complete artificial fertilisers failed to produce a similar effect. The farmyard manure

appeared to protect the young plants from immediate eelworm invasion, but an abundant production of cysts on the roots of the treated plants was evidence that the parasite itself was not greatly affected by this method.

(4) Destruction of the cyst contents and free larvae. Direct control of the root eelworm H. schachtii and especially of its beet and potato strains has been the subject of much investigational work involving a large number and variety of substances. Early attempts to combat "beet sickness" in Germany had such little success that several authors including Kühn (55) and Baunacke (1) came to the conclusion that direct chemical methods were impracticable.

Many compounds such as ammonium salts, formaldehyde and common salt were all claimed to have had beneficial effects in controlling "beet sickness", (Baunacke (1)). Hollrung (43) recommended the use of carbon disulphide, 100cc. of which was poured into holes in the infested soil, 20 cm.deep and 50 cm. apart, and the holes then filled up to prevent evaporation of the liquid. This substance was extensively tested and was reported to be quite effective when used in large quantities. Goffart (34) found that by using calcium cyanide, a satisfactory measure of control was obtained, but concluded that eradication of the parasite would cost about £300 per acre.

Attempts of a physical and mechanical rather than

chemical nature have also been undertaken to destroy H. schachtii in the German beet fields. Kühn (55), and later Fuchs (30), planned to raise the soil temperature to the thermal death point of the nematode by the method of burning "brown coal bricks", but found this ineffective. Flooding of the infested field was suggested, but it was shown that this served merely to spread the parasite (1). A method of deep ploughing after frost evolved by Karpinsky (51) also failed to control the beet eelworm, because of the depth of soil infestation and resistance of the parasite to cold.

When H. schachtii had been identified as a parasite of the potato plant in this country and the seriousness of the disease realised, many attempts to control the nematode were commenced. The first series of field experiments on potato sickness in Britain was recorded by Morgan (67) in 1925. Among the many substances tested were naphthalene, whose action has been discussed before, sulphur, bleaching powder and common salt. Peters (75), in the belief that H. schachtii was more prevalent in acid soils, advocated the use of lime, but Morgan (67) and Smith and Miles (94) showed that no benefit as regards eelworm infection was obtained by the application of lime. Carroll (8) & Edwards (20) both showed that paradichlorobenzene was ineffective. Compounds of iron were tested by several investigators and Hurst and Triffit (45) proved that ferric

chloride and ferrous sulphate, in sufficient concentration, had each a lethal action on H. schachtii larvae, while the presence of ferric oxide in potato root excretion containing cysts delayed hatching. No great benefit was found by these or other workers by the application of these substances to infested soil.

Potash salts have also been used in attempts to control the potato eelworm and in some cases improvements in crop have been noted. In 1935, Blenkinsop (3) reported that in certain areas in Devon and Cornwall H. Schachtii was prevalent in soils having high phosphate and low potash contents. When an unusually large quantity of potash was applied to the soil, a remarkable improvement resulted and the disease almost disappeared. The action of the potash, however, was to restore the phosphate-potash balance in the soil and its presence, like those of other fertilisers, served in this case to increase the resistance of the plant to the effects of eelworm attack.

Hypochlorites and certain other halogen compounds, whose action at low concentrations in stimulating larval emergence has already been noted, are effective in destroying cysts in stronger solutions. Smedley (89) reported that a solution of sodium hypochlorite having 1% available chlorine could dissolve the protective cyst walls and kill the living

contents within half an hour. Application of these substances to the soil is impracticable owing to their instability.

One of the most successful compounds yet found for controlling H. schachtii is phenyl isothiocyanate. Smedley (91) recorded that this substance, and ethyl and n-butyl isothiocyanates, all oily liquids with pungent odours, exerted a very strong toxic effect on the larvae of the worm. The phenyl derivative had the most marked influence on the cysts and at a concentration of 0.001% killed the contents of cysts within 3 days. This chemical exhibited the very unusual action of being more effective against cysts than against larvae, but even so, a solution of 0.02% killed larvae in 7 hours while a solution of 0.004% killed them after 2 days.

For the purposes of a field experiment, the compound was absorbed on talc and incorporated in the soil. A relative application of 2 cwt. per acre provided an increase in the yield of potatoes, and only 74 new cysts were formed, as compared with 655 on the untreated plot. In a pot experiment, where the compound was more intimately mixed with the soil, a similar treatment killed all the cysts. Thus with an application of 2 cwt. per acre complete control was established, when the substance was brought into sufficiently close admixture with the soil. Factors such as its prohibitive cost, however, have rendered the general use of the compound impracticable.

Within the last few years, calcium and ammonium chloroacetates have been widely tested with regard to their action on the potato eelworm both in laboratory and in field experiments. Smedley (90) observed that ammonium chloroacetate in a 1% solution could kill free larvae of the nematode in 15 hours, and when applied at the relative rates of 10, 15 and 20 cwt. per acre to small quantities of infested soil, the cysts from the soil failed to liberate any larvae under the stimulus of root excretion. Cysts in soil treated with calcium chloroacetate at the rate of 5 cwt. per acre or less are not killed, according to O'Brien et al.(74), but emergence of their larvae is definitely delayed and this produces a reduction in the infection and an increase in yield of potatoes grown in the treated soil. Experiments by Edwards (21) showed that no reduction in the cyst content of the soil accompanied the beneficial effect of calcium chloroacetate on the growth of potatoes and on the yield of tubers.

O'Brien et al. (74) also indicated that calcium chloroacetate has a very marked phytocidal action, and when used in control of potato eelworm, it must be applied at least three weeks before the planting of the potatoes. It has the added disadvantage, apparently in common with other substances which have given promising results, of having a relatively high cost.

In field experiments on the control of the potato eelworm, the most extensively tested chemical is undoubtedly calcium cyanamide. Under the name "nitrolim", it was amongst the first substances employed in eelworm control trials by Morgan (67) in 1925, and since then many workers, such as Miles (64) and Carroll (8), have included it in control experiments, and some have advocated its use in practice. Previous to 1935, cyanamide was applied at various rates up to 10 cwt. per acre and although increases in crop were obtained, large numbersof cysts were formed on the roots of the treated plants. The compound was thus acting merely as an additional fertiliser in assisting the plants to overcome the effects of eelworm attack.

In 1935, Hurst & Triffitt (46) showed that when cyanamide is present in the soil at certain concentrations, it is toxic to cyst contents. This toxic action is due apparently to the decomposition products of the compound when it is mixed with soil and not to cyanamide itself. Hurst & Triffitt recommended that the cyanamide should be incorporated as uniformly as possible in soil which is in a dry condition, and that planting of potatoes should be delayed until the phytocidal effects of the compound have worn off. Pot experiments by Hurst & Franklin (47) indicated that, at the rate of 40 cwt. per acre, cyanamide could provide complete protection from

eelworm attack. This conclusion, however, was not borne out in field trials (48), due to incomplete incorporation of the substance in the soil. Edwards (20) showed that 80 cwt. of cyanamide per acre is necessary to prevent an increase in the cyst population of the soil and that 100 cwt. per acre will provide a decrease of 46%.

Calcium cyanamide has one great advantage in that it is normally manufactured on a large scale, and widely used as a fertiliser, and as such its cost is not excessive. Other problems present themselves however, since the very large amount of the substance necessary for eelworm control introduces into the soil a large excess of nitrogen. Furthermore, in common with other substances, there lies in its application the ever-present difficulty of obtaining a uniform and intimate mixture of the compound and the soil.

To this brief review of some of the many methods investigated and substances tested for the control of H. schachtii, it may be added that up till now, none has been used on a large scale by growers. The only safeguard that farmers can adopt, if rotation of crops is not practised, is to prevent the spreading of the disease to uninfested land by planting seed potatoes from which adhering soil, which may contain cysts, has been removed and by other obvious precautionary measures.

Prevention of the spreading of the parasite and other procedures which may be adopted, such as rotation, to ameliorate the effects of the disease are certainly progressive moves, but even at best they are defensive weapons and do not solve the fundamental problem of the control of larval invasion of the rootlets of the host plant.

Organic Silver Compounds

The phytocidal properties of silver have long been known, and in 1910, Vermorel & Dantony (118) suggested its use in the form of a 0.02% silver nitrate soap solution, as a fungicidal spray substitute for Bordeaux Mixture. In 1934, McCallan & Wilcoxon (63), in a systematic survey of the toxic action of elements, found osmium and silver to be the most potent. Recently, Seifriz & Uraguchi (86) stated that silver took the first place in order of toxicity in a list of eleven heavy metals. They also stated that a 0.00005 molar solution of silver nitrate could kill the naked protoplasm of the slime fungus Physarum polycephalum in about 100 seconds.

In 1939, Hovy (44), working in Southern Rhodesia on the tobacco eelworm Heterodera marioni, reported complete control of this parasite using very dilute solutions of organic silver salts, the most satisfactory of which was silver proteinate. The silver ion in strongly dilute solutions of 0.9 - 0.01 mgm. silver per litre is highly germicidal, and, according to Hovy, can exterminate small animal organisms of the size of fish larvae. The two main practical difficulties in the use of silver salts are (a) their photosensitive properties which quickly render solutions inactive, and (b) the adsorption of silver ions on organic matter. Hovy claimed to have overcome these by the use of soluble organic silver

compounds.

Hovy's in vitro experiments showed that 1 mgm. silver nitrate per litre killed all H. marioni larvae in about 1 hour. In field trials silver proteinate was applied at the rate of only 500 gm. per acre and 7% of the treated plants were found to be infected, compared with 90% of the controls, and of this 7% the average degree of infection was less than 5%, whereas that of the controls was 26%. A very desirable feature of this method was the low cost of 21/- per acre. Complete control was achieved in hothouse tests using about three times the above application.

The tobacco eelworm H. marioni does not encyst like H. schachtii, but forms galls on the roots of the host plant from which the larvae are liberated. It would be thought, therefore, that the silver ion would have a similar action on the larval stages of both nematodes.

In order to determine the effect of organically combined silver on the larvae of the potato strain of H. schachtii, to obtain information upon its action on the cyst contents of this nematode, not only in vitro but also in the soil, and to find whether its use might ultimately lead to a practicable means of control, the study of the reactions of the potato eelworm to organic silver compounds was taken up.

A. Experiments with Free Larvae.

Materials.

The principal silver compounds used in the investigations were the proteinate, acetate, lactate, benzoate and nucleinate all of which are water-soluble to a greater or lesser extent. The proteinate by reason of its having the lowest silver content (about 8%) is cheapest.

Silver proteinate is soluble up to 1:2 in water, and a 1% solution gives with dilute hydrochloric acid a precipitate of unaltered proteinate which redissolves on warming. With a 5% solution of sodium chloride - a compound commonly present in soils, especially those of a light nature situated near the sea - no turbidity is produced by any of the concentrations of silver proteinate most frequently used in the investigations even up to a strength of 5%. A clear liquid is also obtained with solutions of less than 2% silver nucleinate and 0.005% silver acetate, benzoate and lactate. Concentrations of 0.01% and above of these three last named salts give an immediate turbidity or precipitate with sodium chloride.

(1) In Vitro Experiments.

Freshly hatched Heterodera schachtii larvae were obtained by storing a large number of potato eelworm cysts in a beaker with potato root excretion. The beaker and its contents

were kept in an incubator at 20°C. for a week or ten days, after which time an abundant supply of larvae was available.

The contents of the beaker were poured through a 0.1 mm. seive which retained the cysts and allowed the larvae to pass through with the root excretion. When this larval suspension was thoroughly agitated, either by stirring or by blowing air through, it was found that a fairly uniform number of larvae could be withdrawn in a given volume of the suspension.

The effect of solutions of silver compounds on the larvae was investigated by introducing 2 cc. of twice the required concentration of the salt into watch-glasses containing 2 cc. of the larval suspension, and immediately agitating the mixture with a needle to obtain quickly a uniform solution of the correct concentration.

The larvae were observed at intervals by means of a binocular microscope, and the time taken for each solution to kill all the larvae was noted and taken as a measure of the toxicity of the solution. This method was considered, under the circumstances of these experiments, to be more practicable than that involved in the determination of Trevan's (105) "median lethal dose" which would have necessitated the counting of so many individual larvae, that only small and therefore very unrepresentative samples could have been used.

Unfortunately, it is not always possible to determine

at a glance whether larvae are dead or merely in a quiescent state. According to Lapage (57), many of those who have studied the problem in other nematodes have taken failure of the larvae to move, either as a result of or without the action of a stimulus, as an indication that they are not alive. Normally under suitable conditions, the larvae are in active motion, but even when most of them are active, it often happens that a few remain motionless although apparently not dead. They may lie in this state for a relatively long period, and then for some undiscovered reason commence active movement once more. At the onset of adverse conditions, such as a drop in temperature which sometimes may be only very slight, larvae quickly become stiff and straight and lie motionless at the bottom of the watch-glass, but in almost all cases activity soon returns when the original temperature is restored.

In the living freshly-hatched first-stage larva, the posterior part of the body containing the intestine appears quite dark on account of the reserve food material stored there. From the anterior end to a point about one third of the length of the larva, the body appears, on low power microscopic examination, to be quite colourless and almost clear. Baunacke (1) noted a difference between larvae in this condition and those which had been killed, and stated that "if the third part of the body at the oral end, which in the living

larva is almost completely hyaline, is now granulated, it is a sure sign of death having taken place".

Furthermore it was found that some of the concentrations of silver compounds produced in the larvae a swelling like an air bladder at a point about the middle of the larva or slightly nearer the oral end, whereby the body wall was distended and the body contents in that region slightly displaced. The appearance of such a larva gave the impression of a plant cell which had been plasmolysed, but this condition was not reversible. Although it is known that nematodes can suffer extreme distortions in shape and yet remain alive, it was ascertained that neither larvae in this state, nor those fulfilling Baunacke's description of a dead larva, were successful in producing cysts on the roots of potato plants.

These criteria, therefore, were employed to estimate the period taken for the various solutions to kill larvae of H. schachtii. As an additional test, after the treated larvae were considered as dead, they were washed, together with a quantity of potato root excretion, into pots of sand in which seed potatoes had been planted, since the ultimate standard is whether or not the larvae will attack potato rootlets. If no cysts were found when the plants had died down, it was assumed that the larvae were dead when applied to the pots.

The experiments on the effect of silver compounds on

the larvae of H. schachtii were repeated using larval suspensions in water instead of root excretion. The method employed to obtain a water suspension was to allow all the larvae in a root excretion suspension to settle on the bottom of the beaker, pipette off most of the liquid and refill the beaker with distilled water. This was repeated about three times, after which it was considered that a satisfactory water suspension had been obtained. An attempt was made to modify this method by the use of a separating funnel, but unsatisfactory results were obtained owing to the tendency of the larvae to adhere to the sides of the funnel.

As well as employing the organic silver compounds mentioned, silver nitrate, mercuric chloride and certain other substances were also used for comparative purposes. Most of the silver compounds were tested at strengths of from 1% to 0.000001%. The experiments were repeated several times at an average temperature of 18°C. and for every concentration two watch-glasses were employed, containing suspensions of equivalent numbers (usually about 200) of larvae. Slightly differing results were obtained in some cases, more especially with the weaker solutions. The average time taken for the different solutions to kill potato eelworm larvae were thus found and noted on the tables which follow.

Experimental Results

The experiments whose results are noted below (Table I.) indicate that silver compounds have a marked toxic effect on the larvae of H. schachtii contained in potato root excretion, and this action is evident even at relatively low concentrations. The more concentrated solutions act in a very

Table I.

Average time in hours taken by solutions of silver compounds to kill potato eelworm larvae in root excretion suspensions.

Concn. (%)	Average time in hours taken to kill larvae by:-					
	Prot.	Acet.	Benz.	Lact.	Nucl.	Nitrate
1	½	½	½	½	25	½
0.5	1	-	-	-	18	-
0.1	4	¾	¾	¾	9	¾
0.05	5	1	1	1	13	-
0.01	8	4	4	5	20	5
0.005	10	6	7	7	22	7
0.001	15	9	10	10	30	10
0.0005	18	13	14	14	∞	15
0.0001	∞	18	18	18	∞	17
0.00005	-	∞	∞	∞	-	∞
0.00001	-	∞	∞	∞	-	∞

short time. In most of the 1% solutions, after 10 - 15 mts. many of the larvae may still be in active motion, but the movement gradually becomes slower and more lethargic and finally ceases. At this stage, the larvae usually lie in a slightly curved position or in some form other than the stiff and straight shape which they eventually assume after about 30 minutes. That part of the worm which is normally clear then becomes darker and granular in appearance and in this condition the larvae were considered to be dead.

With the more dilute solutions, the course of the action is the same, although each stage takes a correspondingly longer time. It is interesting to note that after 1 - 1½ hours, the larvae in certain of the weaker solutions (0.001% proteinate and 0.0001% acetate, benzoate and lactate), were observed to be in a more highly active state than the control larvae to which water had been added in place of the silver solutions. In most cases this hyper-normal agility was not maintained for long, and eventually normal activity returned, to be followed by a gradual slowing down, until movement became scarcely perceptible and finally ceased.

When the larvae were considered to be dead, they were transferred to pots of sand in which sprouted potato tubers had been planted, but no new cysts were found on the roots of the resulting plants. On the plants inoculated with larvae

considered to be alive, including the control larvae, an average of 40 cysts was found per plant. This verified that the action of the silver compounds had been definitely toxic to the larvae and that the criteria for the determination of larval death were reliable.

It is evident, from Table I., that there is a marked similarity in the times of action of silver acetate, benzoate, lactate and nitrate, which are at first sight all more effective than silver proteinate. When the silver content of each of the substances (Table II.) is taken into account, however, it may be seen that the lethal effect which they exert corresponds directly to the amount of silver in the solutions. The acetate, nitrate, benzoate and lactate all contain a fairly high percentage of silver - 64.7, 63.4, 58.4 and 54.8 respectively. These figures are all in the same region, and this explains the similarity in the action of these compounds. The silver content of the proteinate is only 8%, and consequently the effect of this substance at similar concentrations is less than those of the others.

Any differences which there are in ionisation in these compounds do not seem to be of great consequence, for silver nitrate which is highly ionised has the same relative effect as silver proteinate which is not ionised to such a large extent. The toxic influence of solutions of the five

Table II.

Silver content of solutions used (mgm.per litre).

Concn. (%)	Silver content (mgm.per litre)					
	Prot.	Acet.	Benz.	Lact.	Nucl.	Nitrate
1	800	6470	5840	5480	2000	6343
0.1	80	647	584	548	200	634.3
0.05	40	323.5	292	274	100	317.2
0.01	8	64.7	58.4	54.8	20	63.43
0.005	4	32.35	29.2	27.4	10	31.72
0.001	0.8	6.47	5.84	5.48	2	6.343
0.0005	0.4	3.235	2.92	2.74	1	3.172
0.0001	0.08	0.647	0.584	0.548	0.2	0.6343
0.00005	0.04	0.3235	0.292	0.274	0.1	0.3172
0.00001	0.008	0.0647	0.0584	0.0548	0.02	0.0634

compounds mentioned above is thus dependent on their total silver content, and practically unrelated to the acid radicles.

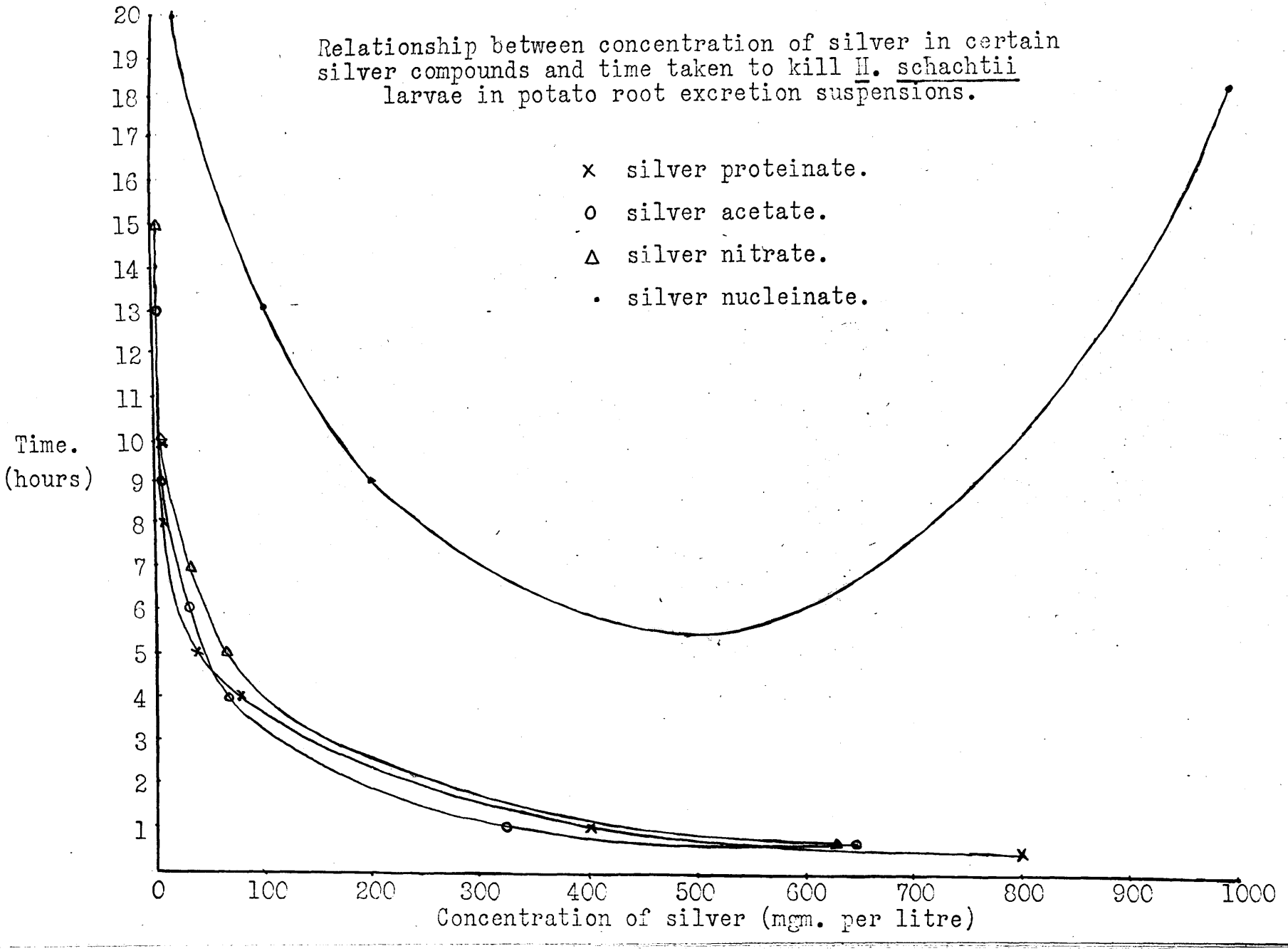
Silver nucleinate, on the other hand, exhibits the curious feature of being more effective at certain lower concentrations, e.g. 0.1%, than at higher ones, e.g. 1%. This substance, which contains 20% silver, does not appear to bear much relationship to the other compounds with respect to its action on eelworm larvae. In this case, the metal may be so closely bound to the acid radicle, that the effect of the

silver is thereby diminished.

The similarity in time of action of certain different solutions with equivalent silver contents is illustrated by the graphs on p.71a which show the close agreement between silver proteinate, acetate and nitrate, and corresponding graphs for silver benzoate and lactate conform to these in a significant manner. From these graphs it may also be noted that the greatest difference in time of action for a particular silver concentration of any of these compounds is about one hour, a divergence which may be well accounted for by experimental variation.

The most effective concentration of silver nucleinate appears, from the graph, to be in the region of 0.25%. The minimum concentration of this substance to have an effect on the larvae has a silver content of 2 mgm.per litre.

The other compounds, however, are active in much weaker solutions and even 0.4 mgm. silver per litre, i.e. 4 parts in 10,000,000, will bring about the death of eelworm larvae in root excretion within 18 hours. From the experiments performed, this value is at the limit of the effective range of the element in potato root excretion. H. schachtii larvae in solutions more dilute than this were observed over a period of 7 days, and even at the end of this time, they were, like the control larvae, in active motion.


Relationship between concentration of silver in certain silver compounds and time taken to kill H. schachtii larvae in potato root excretion suspensions.
× silver proteinate.
o silver acetate.
Δ silver nitrate.
• silver nucleinate.
Time. (hours)
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
100
200
300
400
500
600
700
800
900
1000
Concentration of silver (mgm. per litre)

The difference in times of action of silver solutions on larvae contained in root excretion and water suspensions is evident by a comparison of Tables I. & III.

Table III.

Average time in hours taken by solutions of silver compounds to kill potato eelworm larvae in water suspensions.

Concn. (%)	Average time in hours taken to kill larvae by:-					
	Prot.	Acet.	Benz.	Lact.	Nucl.	Nitrate
1	1	-	-	-	18	½
0.1	2	1	1¼	1	10	1
0.01	3½	2	2½	2	8	1½
0.001	4½	3½	3½	3½	12	3
0.0005	7	-	-	-	-	-
0.0001	36-∞	7	7	7	18	6½
0.00005	∞	18	22	18	-	20
0.00001	∞	48-∞	∞	∞	∞	60-∞
0.000001	-	∞	∞	∞	∞	∞

The periods necessary for silver to manifest its full toxic effect were much shorter when the treated larvae were in a water suspension. To give but one example - 0.001% proteinate killed larvae in root excretion in 15 hours, but in water this time was reduced to 4½ hours. This decrease in time was observed in all cases except in some of the higher

concentrations (such as 0.1%) of the acetate, benzoate, lactate, and nitrate, where slightly increased times were noted, although the reason for this is not clear. Not only were the times of action generally lowered, but certain concentrations which had no apparent effect on the larvae when root excretion was present now showed lethal properties. It was found extremely difficult to obtain constant figures for the 0.0001% proteinate and 0.00001% acetate and nitrate solutions and the average results are indicated as on Table III. It was also noted that 0.00001% benzoate and lactate solutions killed about 50% and 30% of the larvae respectively within one week. The range of the effective concentrations is thus increased when root excretion is absent, so that the activity of the solutions is maintained even where the silver content is as low as about 0.06 mgm.per litre, i.e., 6 parts per 100,000,000.

In water suspensions of larvae, silver nucleinate again showed its peculiar effect of being more potent at certain intermediate strengths. The various periods of time necessary for the different concentrations to act on the larvae were, however, decreased, and the effective range of silver increased, in the absence of root excretion, with this substance also.

Other compounds which have been employed to control potato eelworm, or whose action on the nematode has otherwise been investigated were also used in experiments to determine

their effect on the larvae. This was compared, where possible, with that of the silver compounds, to discover whether larvae were more susceptible to the effect of silver than to the toxic properties of other substances or elements.

The influence of mercuric chloride on the larvae, both in root excretion and in water, was investigated and the results noted on Table IV. and compared with those given by

Table IV.

Average time in hours taken by mercuric chloride and silver nitrate solutions to kill potato eelworm larvae.

Concn. (%)	Mercuric chloride			Silver nitrate		
	Mercury mgm./ litre	Time taken (hours). In root excn.	Time taken (hours). In water.	Silver mgm./ litre.	Time taken (hours) In root excn.	Time taken (hours) In water.
1	7386	1½	2	6343	½	½
0.5	3693	-	2½	3172	-	-
0.1	738.6	3	5	634.3	¾	1
0.05	369.3	5½	7	317.2	-	-
0.01	73.86	9	12	63.43	5	1½
0.005	36.93	15	15	31.72	7	-
0.001	7.386	40	18	6.343	10	3
0.0005	3.693	∞	20	3.172	15	-
0.0001	0.739	-	∞	0.634	17	6½
0.00005	0.369	-	-	0.317	∞	20
0.00001	0.0739	-	-	0.0634	-	60 - ∞

silver nitrate. Both these salts are highly ionised and, assuming that the part played by the anion in the action is comparatively small, the relative toxic effects of silver and mercury were found.

From Table IV. it can be seen that mercuric chloride, although possessing a high mercury content, is not such an effective lethal agent as silver nitrate, at least in its action on H. schachtii larvae. Further, since this action in the case of silver is reasonably constant, whether the element is inorganically combined as in the nitrate or organically combined as in the proteinate and the other compounds used, and since the toxic principles concerned are silver and mercury, it may be deduced that H. schachtii larvae are more susceptible to the effect of solutions containing the former element than to the effect of solutions with an equivalent content of the latter.

The presence of root excretion was found to influence the action of mercury, although its effect is not so evident as with silver. At higher concentrations, mercury appears to act more slowly in water suspensions of larvae than when root excretion is present, but at lower strengths the root excretion has the effect of protecting the larvae, so that the range of active concentrations of mercury is, like that of silver, decreased in presence of the excretion. The critical toxic

concentration of mercury is about ten times greater than that of silver both in water and in root excretion suspensions of larvae.

Organically combined mercury was shown to produce an effect similar to that of the inorganically combined element. Agrosan-G, a seed disinfectant containing 1.5% mercury in organic combination, was used at concentrations of 0.5% and 0.05% with a root excretion suspension of larvae. These solutions, whose mercury contents were almost the same as those of 0.01% and 0.001% mercuric chloride, acted in 7 hours and 100 hours respectively, periods comparable to the times of action of the corresponding mercuric chloride solutions which are 9 hours and 40 hours. (The difference in potency of the solutions which act in 100 hours and 40 hours is not really so great as is indicated at first sight, since the concentration of mercury in each case is near the effective limit of the element and the experimental variations at this point tend to be greatly exaggerated.)

The effect on the larvae of calcium chloroacetate, which has been extensively tested in H. schachtii control experiments, was also investigated. A 10% solution, filtered free from insoluble matter, caused the larvae to curl up and shrink after 3 hours, and after 48 hours to become straight and quite colourless. A solution of 1% strength was found to kill

the larvae within 72 hours.

(2) Pot Experiments.

Large porous pots of 10 inch diameter were filled with eelworm-free sand, whose water content had been determined and which had received complete artificial fertiliser at the rate of 15 cwt. per acre. Suspensions of 10,000 larvae of the potato strain of H. schachtii in equal volumes of potato root excretion were poured on each of the pots. The larval suspensions were estimated by agitating the liquids, withdrawing 2cc. samples and counting the larvae present in them.

In order to ascertain whether the effect of silver proteinate on the larvae of H. schachtii, which was found from in vitro experiments to be considerable, was maintained when the action took place in a medium such as sand, the compound both as a solution and in powder form was applied to the inoculated pots immediately after the larvae had been introduced.

The strengths of the solutions used varied from 0.25% to 0.0025%, and 100cc. of each solution was spread by means of a pipette over the surface of the sand in each pot. It was calculated that the concentrations of proteinate in the sand would, in virtue of the moisture content of the medium, range from 0.05% to 0.0005%. Before application as a powder, the proteinate was mixed with a small quantity of very fine sand,

in order to ensure a more even spread. The quantities of powder and of solution applied produced in each case practically the same range of concentration in the sand.

Sprouted Epicure tubers which had been previously washed carefully were then planted in the pots, and when the growth of the resulting plants had ceased, the sand and roots in each pot were dried and sieved to remove small stones, tubers and larger portions of root. Both these fractions were shaken with water, as in the flotation method of cyst extraction elaborated by Morgan (67), and the cysts which came to the surface decanted on filter paper. This procedure was repeated six or seven times, or until no cysts were observed on the surface of the water in the extraction flasks, after which the extracts were dried, shaken on white squared paper and the number of cysts counted for each pot.

Experimental Results.

The average numbers of cysts formed after the application of various concentrations of silver proteinate are shown on Table V. Some variations were observed, but in general there was a tendency towards the production of a smaller crop of cysts in presence of increasing concentrations of silver proteinate. The main conclusion that can be drawn from the experiment is that even a solution of 0.05% silver proteinate,

Table V.

Average numbers of cysts formed in presence of different concentrations of silver proteinate.

	Quantity applied per pot	Concentration in sand(%)	Number of cysts formed per pot
Powder,	0.25 gm.	0.05	344
	0.05 "	0.01	510
	0.025 "	0.005	400
	0.005 "	0.001	700
	0.0025 "	0.0005	445
Solution,	100cc. 0.25 %	0.05	273
	" 0.05 "	0.01	434
	" 0.025 "	0.005	404
	" 0.005 "	0.001	464
	" 0.0025"	0.0005	629
Control,	-	-	804

which can in vitro kill all eelworm larvae in root excretion suspensions in 5 hours, under the conditions of a pot experiment can effect only a reduction of at most 66% of the larval content of inoculated sand, assuming that equivalent numbers of larvae will form equivalent numbers of cysts. Some of the other weaker solutions produced a correspondingly smaller

reduction, which could not be compared to their effect under in vitro conditions.

It may also be seen that application of the compound as a solution is more effective than when the proteinate is spread in powder form.

From a further experiment conducted along the same lines, using the proteinate only as a solution at concentrations between 0.05% and 1%, little could be deduced, because for some inexplicable reason very little infection resulted even in the control pots. To each of these, 10,000 larvae were added but an average of only 28 cysts was found per pot. The average numbers of cysts produced after treatments with 1%, 0.2%, 0.1% and 0.05% were respectively 0.5, 4, 1 and 0, and thus no definite conclusions could be drawn. Another experiment performed at the same time (mid-summer) also gave practically no infection.

Discussion

It has been shown that solutions of certain organic and inorganic silver compounds, such as the proteinate, acetate, benzoate, lactate and nitrate have a very strong toxic effect on larvae of the potato strain of H. schachtii, the time taken by the various solutions to kill the larvae being taken as a measure of their relative toxic potency. That the larvae were

killed by the various treatments was evidenced by the facts that their appearance, subsequent to treatment, agreed with Baunacke's description of a dead H. schachtii larva, and that they produced no cysts on the roots of potato plants.

It was also demonstrated that with one exception the toxic effect experienced by the larvae varied with the silver content of the solution and was independent of the nature of the original compound. With all concentrations of silver the action took place in a comparatively short time, and even in solutions of critical toxicity it was complete, in almost every case, within 24 hours. Thus not only is the influence of the silver definitely toxic, but its action is relatively quick.

Larvae in certain of the more dilute solutions, such as 0.001% proteinate and 0.0001% acetate, were observed to be in a temporary state of hyper-normal agility. This condition may be connected with a phenomenon described by Baunacke (1), and termed by him "flight movement". He observed that when larvae of the beet strain of H. schachtii were exposed to a sudden rise in temperature to 28°C., i.e. above the point of their normal maximum agility, they exhibited quick jerky movements, and in order to produce this increase in motility and provide the necessary energy, there was a rapid consumption of the reserve food material in the larvae. As an explanation of the phenomenon, it was suggested that eelworm larvae which may

be suddenly overtaken by conditions which imperil their safety, as for instance when exposed to the action of wind or sunlight, make use of these movements in order to escape to cooler or less dangerous regions of the soil. A similar explanation may also hold good for the temporary hyper-agility shown by the larvae in presence of certain concentrations of silver salts, only in this case it is the reaction of the larvae to chemicals before the toxic action of the latter is fully manifest.

The silver solutions act in the same manner upon larvae in water and in root excretion suspensions. The presence of root excretion, however, slows down the action in most cases, and appears to have a protective effect on the larvae, so that the critical toxic concentration in a root excretion suspension is higher than in a water suspension, the respective figures being about 0.4 and 0.06 mgm. per litre. The reason for this is probably the tendency for silver ions to be adsorbed on particles of organic matter present in the root excretion. The phenomenon, however, is not peculiar to silver, since the action of mercury at lower concentrations is also limited to some extent in root excretion, although not quite so much as that of silver.

Freundlich (29) has suggested two processes in the precipitation of proteins by heavy metals, the action which is probably involved in the toxicity of these elements. First, a

loose and usually reversible adsorption compound of the protein and the heavy metal is produced, which leads in the course of time to the formation of true and irreversible heavy metal salts. Seifriz and Uraguchi (86) consider that, although toxicity, as a physiological process, involves more than adsorption it is probable that adsorption of metal cations by protoplasmic proteins plays the primary rôle in the toxic action of heavy metals on protoplasm. They found a direct relationship between the adsorption on blood charcoal and the toxicity of five of the heavy metals, including silver and mercury. Besides providing an explanation of the toxic action of silver and other heavy metals on protoplasm, this work indicates that the reason for the limitation of the toxic effect of silver and mercury in presence of potato root excretion is adsorption of the metallic ions on protein and other organic particles in the medium.

Seifriz and Uraguchi (86), in their work on the toxicity of heavy metals to protoplasm, found that a 0.05 molar solution of silver nitrate had an immediate toxic effect on the naked protoplasm of the slime mould Physarum polycephalum. On account of the highly resistant cuticle of the larvae of H. schachtii, solutions of a similar silver content required a longer time to act on the nematodes, but killed them in 1 hour. The above workers state that a 0.000005 molar silver nitrate

solution acted on the slime mould in more than 30 mins., but do not indicate whether this concentration was found to be the effective limit. With H. schachtii larvae in a water suspension a solution with a corresponding silver content (0.54 mgm. per litre) acted in 7 hours.

However low the active strengths and critical concentrations found in the present investigation may be, the values noted by Hovy (44) are still lower. This worker mentions the strong germicidal properties of solutions of even 0.01 mgm. silver per litre, but does not state whether it was sufficient to kill nematode larvae. Hovy also records that, in vitro, solutions of 1 mgm. silver nitrate per litre (0.63 mgm. silver per litre), killed all H. schachtii larvae in about 1 hour. From the results of the present investigations, it may be deduced that a similar solution will kill potato eelworm larvae in water in 6-7 hours, and in root excretion in 17-18 hours. Again, by comparison, the concentration of silver necessary to kill the larvae of H. schachtii in a water suspension in 1 hour is between 500 and 1000 times greater than the corresponding concentration for H. marioni.

The greater toxic activity of silver solutions noted by Hovy and their potency at strengths lower than those observed in the experiments described above, may be due to one or both of the following factors: (a) the possible difference between

the conditions in Southern Rhodesia under which Hovy worked and those of the present investigations, and (b) the fact that Hovy employed another although closely related eelworm. It is hardly likely that dissimilar environmental conditions can fully explain such vast differences in the toxicity of silver proteinate in the two investigations, nor can they be wholly attributed to the slight variations which occur in the silver content of different samples of silver proteinate. It must be considered therefore that, although direct evidence is lacking, larvae of the potato strain of H. schachtii are not so susceptible to the toxic action of silver as those of the tobacco eelworm H. marioni.

The toxic influence of solutions of equivalent silver contents was seen to be practically constant for all the silver compounds employed in this investigation with the exception of silver nucleinate. This salt bears no relationship to the others with respect to its action on eelworm larvae, apparently because the silver is strongly bound in the molecule, and further increases after a certain concentration has been reached bring about a decrease in toxicity. A similar phenomenon was found to occur when bunt (Tilletia tritici) spores were killed by certain intermediate concentrations of copper sulphate, while solutions either stronger or more dilute had no lethal action on the spores. According to Thomson (103) this happens

because in killing the spores the copper solution neutralises the electric charges on particles of the protoplasmic colloidal system causing coagulation. At higher concentrations, however, the colloidal particles lose their own charge but take up another and so remain stable, with the result that no toxic action takes place. This re-formation of a sol from a gel or coagulated sol is called peptization.

The toxicity of silver compounds to the larvae is much greater than that of certain other substances, such as mercuric chloride and the chloroacetates, which have been tested with a view to controlling the parasite. Even substances with a low content of silver, such as the proteinate containing 8% silver, have a stronger effect than mercuric chloride which contains 73.8% mercury, illustrating at the same time, that silver is relatively more toxic than mercury. Although mercury has been used in various forms in experiments to control potato eelworm, no record of its action on the free larvae appears to have been published.

Smedley (91) (90) reported that phenyl isothiocyanate, at a concentration of 0.02%, killed potato eelworm larvae in 7 hours and that a 0.004% solution killed them in 2 days, while 1% ammonium chloroacetate brought about their death in 15 hours. O'Brien et al.(74) stated that 1% calcium chloroacetate had no toxic effect on H. schachtii larvae. Contrary to this however

in the experiments previously described, this compound at the same concentration was found to kill larvae within 72 hours. Basing a comparison on the strengths of these substances and their corresponding times of action, it can be seen that any of the silver compounds is superior in toxic effect to the chloroacetates, and that phenyl isothiocyanate, although giving results more comparable with those of silver proteinate, does not reach the standard of this silver compound.

The very strong toxic influence exerted on potato eelworm larvae by the silver compounds in experiments carried out in vitro, was not maintained in pot tests. It is highly improbable that the inefficiency of concentrations of silver proteinate which are known to have the power of killing larvae, even with root excretion present, was due to most of the larvae having penetrated the potato rootlets before the compound had time to act. It is impossible to conceive of several hundred larvae invading the limited system of root primordia of a sprouted potato tuber in a few hours. The margin of experimental error in the estimation of 10,000 larvae may have been responsible for some of the variations in the numbers of cysts formed in equivalent treatments, but it cannot explain why so many were produced in presence of a relatively high concentration of a toxic substance. It must be assumed, therefore, that the compound both as a powder and in solution was confined

mostly to the upper layers of the sand, at least for a considerable time after application. In this way, many of the larvae would be killed while others would make their way by active movement to those parts of the pot to which the proteinate had not yet penetrated, there to remain until such time as they were induced to attack a rootlet in their neighbourhood.

It may thus be seen that the concentrations of silver proteinate necessary to kill eelworm larvae and to control larval infection in pot experiments, even using sand which excludes many of the complex factors introduced by soil, are very much greater than those which were found to be effective in vitro. They were also greater than those found necessary by Hovy (44) in field experiments to control the larvae of H. marioni. Hovy determined that 0.1 gm.per plant or 500 gm. per acre gave a control ratio of 1:64 compared with untreated plants, and found that 0.3 gm.per plant provided the required 100% control. In the experiments previously described it was found that 0.25 gm.of the compound per plant, when applied as a powder and in solution, gave respectively 57% and 66% control.

It is possible that other methods of application, such as mixing the compound with the sand before potting either before or after the sand has been inoculated, may provide results more in agreement with those of the watch-glass tests. The conclusions from the more easily practicable method which

was employed in the experiments would, however, still stand and can be better compared with the results of Hovy's investigations.

B. Experiments with Cysts.

(1) In Vitro Experiments.

However encouraging the results of the action of experimental substances on the larvae of Heterodera schachtii may be, these compounds must produce a permanent effect on the contents of the cysts of the nematode if lasting control of the disease is to be obtained, for the cysts are the centres of infestation in the soil and agents by which the disease is spread to other fields and new districts.

The cystic stage in the life history of the parasite is a protective phase, first of all for the fertilised eggs and then as maturity proceeds for the embryonic larvae. It must therefore be expected that whatever toxic effect may be produced by a given substance on the larvae, its action on the cyst contents which are protected by an efficient wall will be greatly diminished. Curiously enough, however, at least one substance - phenyl isothiocyanate - has been shown to produce a stronger effect on the cyst contents than on the free larvae (91). Apart from possible diffusion of water soluble substances through the cyst wall, the only means by which toxic compounds can reach the contents is by the small aperture of the neck canal.

Materials and Methods.

Cysts. The cysts used in all these investigations came from the same source - the Warren field, Jameston Farm, Maidens, Ayrshire - a field in which potatoes have been grown annually for many years up to and including 1940. The variety grown in this as in other fields in the district was Epicure.

Extraction of cysts was performed by the Morgan flotation method. About one hundredweight of the infested soil, several pounds at a time, was shaken up with water and the floating material decanted and collected. The bulk extracts were dried and then sieved, the fraction between 1 mm. and 0.1 mm. being retained. This fraction, which contained practically all the cysts which had been extracted, was cleaned by placing it on a sheet of stiff paper held so as to slope into a petri-dish and collecting the cysts, most of which roll down more quickly than the rest of the material. In this way, a fairly concentrated sample of potato eelworm cysts was obtained.

Potato Root Excretion. It is known that first-stage larvae are normally liberated from potato eelworm cysts only under the influence of the excretion which is exuded from potato roots. A few larvae do emerge when the cysts are stored in water, but the number is incomparably smaller than the "hatch" in root excretion. It is indeed unfortunate that the chemical composition of this secretion is as yet unknown and

that no method has been evolved whereby it can be standardised. Another difficulty connected with root excretion is that even the most potent solution, when stored for a time, loses some of its strength.

The method employed to obtain root excretion in quantity was to grow potato plants in light soil contained in large filter funnels and allow a volume of water to soak through the soil at a time when the roots were growing actively. This liquid was collected and poured through again, the process being repeated several times. About a week later, a fresh quantity of water was poured through and the procedure carried out as before. The final extracts were combined, a volume of about half a litre being obtained from each plant, and the liquid filtered before use.

Emergence of Larvae.

The normal course of larval emergence, as far as has been found in these experiments in which a number - usually 50 - of potato eelworm cysts were treated with potato root excretion, is that the first larvae to be liberated are observed after a few days. The rate of emergence quickly increases to a maximum and then, rather more slowly, begins to decrease until finally it practically ceases. Liberation of larvae does not as a rule stop completely, but the numbers which emerge after this point has been reached are negligible. The cause of this

is apparently not to be found in the root excretion, for even although the cysts are transferred regularly to a fresh volume of the liquid, the normal rate-of-hatching curve is produced.

All the phases in the progress of larval emergence may vary according to differences in the cysts, the potency of the root excretion and other similar factors. Consequently some samples may cease hatching after 20-30 days, while others may liberate significantly large numbers of larvae even up to 40 days. In some cases, particularly with newly-formed cysts, it was found that there was more than one maximum in the rate of hatching, and that emergence of significant numbers of larvae continued for about 80-90 days.

Moreover some authors, such as Triffitt (111) and Gemmell (32) mention a periodicity in the hatching of larvae, having noted that fewer larvae emerge in autumn and winter from cysts in contact with potato root excretion and that the larval output is greatly increased in spring.

It is necessary, therefore, in an examination of relative larval outputs to compare directly only those from cysts taken from the same sample and exposed at the same time of year to root excretion from the same stock. Another factor must also be borne in mind. When the sample of cysts is extracted from eelworm infested soil, the individual cysts may be of different ages and may contain different numbers of

viable eggs. This fact accounts for differences in hatching within the sample itself. In comparing the effects on the cyst contents of the different treatments which will be described later, the emergences of larvae from three replicated sets of 50 cysts each were examined in an attempt to reduce internal variations of the sample to a minimum, and this method proved to be satisfactory in almost all cases.

Experimental Methods.

Experiments to determine the effect of solutions of organic silver compounds on H. schachtii cyst contents were performed, (a) by pre-treating the cysts in solutions of the requisite concentrations of the substances, (b) by treating the cysts with silver solutions in presence of potato root excretion, and (c) by treating cysts actually contained in samples of infested soil by application of solutions of the substance under investigation to the soil.

From these experiments a fairly comprehensive picture of the action of organic silver compounds on the cyst contents was drawn, and tests along similar lines may be devised to investigate the toxicity to potato eelworm of any other water soluble compound.

(a) Pre-treatment Experiments. The principle of this method is to bring the cysts into contact with the experimental substance for a definite length of time, and then to transfer

them to potato root excretion, in order to determine the effect of the substance.

About one thousand potato eelworm cysts from the same sample were counted out into several watch-glasses, into each of which was introduced 2 cc. of the required solution of the silver compound. Silver proteinate, acetate, benzoate, lactate, and nucleinate were used at strengths ranging from 1% to 0.0001%, and the cysts were kept immersed in these solutions for periods up to 6 weeks at a constant temperature of 20°C.

At certain intervals three sets of 50 cysts were counted out from the treatment reservoirs and placed in drops of water in clean watch-glasses. This water served to wash away adhering traces of the silver solutions and was removed after a few minutes by drying off with filter paper and immediately replaced by 2 cc. potato root excretion. Each set of three watch-glasses, containing similarly treated cysts, were placed on moist filter paper in a large petri-dish, and stored in an incubator at 20°C. The atmosphere in the petri-dishes was kept damp by reason of the moisture in the filter paper and evaporation from the watch-glasses was thus minimised.

The watch-glasses were examined periodically by means of a binocular microscope, and the number of larvae in each counted. At certain intervals, especially when the rate of hatching was at a maximum and when the numbers of larvae

counted were very great, the root excretion containing all the larvae present was withdrawn by means of a fine pointed pipette and the moisture adhering to the cysts dried off. A fresh quantity of root excretion was then placed in the watch-glasses and the hatching process continued. This method of removing the larvae and changing the root excretion not only facilitates the counting of large numbers of larvae, but tends to induce the maximum output. Examinations of the relative "hatches" were carried out until the larval emergence had for all practical purposes ceased.

In cases where no larvae were found to have emerged from treated cysts, absence of observed hatching might have been due merely to a delay in emergence and not to the death of the cyst contents. When active emergence from those cysts which had produced larvae had ceased, therefore, the cysts used in the test were dried, stored for a certain period and then replaced in potato root excretion, to determine the numbers of fresh larvae, if any, which had hatched.

(b) Treatment of cysts in presence of root excretion. These experiments were performed to determine whether the combination of root excretion and silver solution would cause the larvae to emerge from the cysts and then be killed by the action of the silver, or whether the toxic effect of this element would be nullified by the root excretion.

Series of watch-glasses, each containing 50 eelworm cysts were set up in petri-dishes in the manner described before. Into a number of the watch-glasses was placed 1.8 cc. potato root excretion together with 0.2 cc. of a silver proteinate solution whose concentration was ten times greater than the desired strength, and the resulting liquid stirred so that the requisite concentration of the silver compound was uniform throughout. The same procedure was carried out with other cysts, using water in place of root excretion. The control series comprised cysts immersed in 90% root excretion (the concentration of this substance in the mixtures with silver proteinate), 100% root excretion and water. The concentration of the silver proteinate, both in water and in root excretion, covered the range between 0.1% and 0.001%.

The cysts were kept in these solutions for 21 days during which time they were examined at regular intervals and the larvae counted. All the solutions were then drawn off, fresh root excretion introduced into the watch-glasses and the emerging larvae counted for a further period until hatching had ceased.

(c) Treatment of cysts in samples of infested soil. Before practical application of any substance can be made in control of H. schachtii with a degree of certainty as to its effect on the nematode, its action on the cysts actually in the soil must

be known.

For this purpose, large filter-funnels each containing 5 lb.air-dried eelworm-infested soil were set up and through the soils were poured 500 cc. 1%, 0.1%, 0.01% silver proteinate solutions and 500 cc. water. This volume was just sufficient to saturate the soils and gave about 3 cc. filtrate in each case.

After 1 day and 1, 3 and 6 weeks, small quantities of soil were extracted from the funnels and the cysts separated out. Three lots of 50 cysts were then taken from each sample, placed in 2 cc. potato root excretion and the larval emergences noted at intervals.

At the same time, similar cysts, i.e. cysts extracted from the same original soil as that in the funnels, were pre-treated for 1 day, 1, 3 and 6 weeks in the filtrates from the funnels and then placed in root excretion, in order to determine whether the solutions still possessed an actively toxic effect after passing through 6 inches of soil.

Together with these experiments and to act as a check on the activity of the solutions on cysts in vitro, similar cysts were pre-treated for the same periods in small quantities of the same solutions of silver proteinate.

The larval outputs from the cysts under examination in these tests were noted at regular intervals, and from time

to time, fresh root excretion was introduced into the watch-glasses by the method previously described.

When the last quantity of soil had been extracted from the funnels after 6 weeks, the soil which remained in each was thoroughly mixed, and after samples of cysts had been separated out for "hatching" purposes, the soils were potted and a sprouted tuber planted in each pot. Portions of the roots of the resulting plants were examined at various times, and the amount of infection in each case estimated by a method which will be outlined in the description of the pot experiments.

Experimental Results.

(a) Pre-treatment Experiments.

From a preliminary test designed to ascertain the range of concentration over which the silver compounds were effective in killing the cyst contents of the potato eelworm, it was observed that 0.1% proteinate, acetate, lactate and benzoate controlled larval emergence completely, even after 1 day's immersion of the cysts in the solutions, and that 0.0001% of these substances had no toxic effect even after 6 weeks. A solution of 0.01% proteinate provided a constant reduction in larval output, indicating that the critical concentration of this substance is in this region.

In its effect on the cysts silver nucleinate did not

behave like the other compounds, for cysts immersed in a 0.1% solution of the nucleinate for even 6 weeks were still capable of producing a consistent although much reduced "hatch". The weaker action of this compound on the cyst contents compared with that of the other salts used corresponds with its lower toxicity to the larvae of the nematode. In view of this lack of potency, no further experiments were conducted with silver nucleinate.

A more detailed experiment was performed using the proteinate, acetate, lactate and benzoate of silver, and the results are shown on Table VI. The cysts used were taken from a sample which had been extracted from infested soil a few months before, and which contained cysts formed on the previous season's potato crop.

From Table VI. a correlation can be seen to exist between larval emergence and the strength of solution in which the cysts were immersed, and further, the decrease in "hatch" is proportional to the concentration of silver in the solutions of all the compounds used. It is thus indicated, as in the results of the experiments with larvae, that the efficiency of the solutions is dependent mainly on their silver content. Increase in time of exposure of the cysts to the action of the silver compounds up to a period of 6 weeks, which was the limit of the experiment, also diminishes the

Table VI.

Relative larval emergences from cysts immersed for different periods in solutions of silver compounds.

Silver Compound	Concn.of solns.		Relative larval emergences (% of control)			
	%	Mgm. Silver p.litre	1 day	1 week	3 weeks	6 weeks
Prot.	0.05	40	4.64	0.09	0	0.24
	0.01	8	64.9	46.9	19.25	42.96
	0.005	4	120.5	99.0	87.3	32.3
	0.001	0.8	291.5	191.1	107.4	103.6
Acet.	0.05	323.5	0	0	0	0.04
	0.01	64.7	0.69	0	0	0.04
	0.005	32.35	30.93	0.47	0.03	0.16
	0.001	6.47	66.39	5.93	8.17	11.7
Benz.	0.05	292	0	0	0	0
	0.01	58.4	1.12	0	0	0.2
	0.005	29.2	106.9	13.45	1.63	4.15
	0.001	5.84	200.6	177.2	119.4	99.9
Lact.	0.05	274	0	0	0	0
	0.01	54.8	3.55	1.84	0	0.12
	0.005	27.4	182.1	35.6	4.96	1.41
	0.001	5.48	180.2	54.0	54.48	43.65
Control[x] (water)	-	-	100 (2758)	100 (3419)	100 (3526)	100 (2479)

[x] Actual number of larvae shown in brackets.

larval output from the treated cysts. The action of the time factor is more clearly seen during the first three weeks of storage than later, when there appears a tendency to establish an equilibrium between the magnitude of the "hatch" and the potency of the solution, without regard to the period of immersion.

In spite of some differences in the relative emergences from cysts treated with different solutions whose silver contents were approximately the same, the critical silver concentrations after the various times of storage tend to be similar in the four substances employed, although in some instances silver acetate appears to be the most effective.

Practically complete control of hatching was obtained after storage of cysts for 1 day in solutions containing more than 65 mgm.silver per litre. After 1 week, the critical strength was lowered to about 40 mgm., except in the case of the lactate. After 3 weeks, this figure was further decreased to about 35 mgm. and the critical concentration remained around this amount even after 6 weeks. Lower strengths than those which just control hatching effected a considerable reduction in larval emergence, e.g., a solution of silver acetate containing 6 mgm.silver per litre, which after acting on the cysts for 6 weeks allowed only 11.7% of the normal number of larvae to emerge.

It was observed that after a relatively short immersion in some of the more dilute solutions, cysts liberated an abnormally large number of larvae. This stimulation was particularly evident with a silver proteinate solution containing 0.8 mgm.silver per litre which, after 1 day, induced a "hatch" almost three times greater than that of the control. After 1 week, stimulation, although much reduced, was still given by this solution but after 3 weeks, the output of larvae was practically at the control level.

The stimulation caused by such solutions as 0.005% silver proteinate and 0.001% silver lactate gave place later to a definitely toxic effect, which may be seen by the larval emergences from cysts immersed in these solutions for 6 weeks. This may be explained by the longer time of the action producing a cumulative effect which was toxic to the cyst contents. On the other hand, however, solutions such as 0.001% silver proteinate and 0.001% silver benzoate, which also had an early stimulative action, had no toxic effect even after 6 weeks, when their larval emergences were closely comparable to that of the control. There obviously must be a time between the stimulative and toxic effects of some of these solutions when they will act in the same way as water, and it may be that the two last mentioned solutions would, if allowed to act for longer than 6 weeks, prove toxic to the cyst contents.

After the cysts used in this experiment had been stored in a dry condition for 48 days, they were replaced in potato root excretion, and the relative larval emergences for the "rehatch" are noted on Table VII.

The numbers of larvae liberated were not so great as on the first occasion, but this was quite to be expected. The emergences from the cysts which had been previously treated for 6 weeks were much larger than any of the others and this seems to compensate for their unaccountably smaller "hatch", after the first contact with root excretion.

It was unfortunate that the root excretion used for the second "hatching" was not from the same stock as that used previously, and its strength may have been slightly different from that of the first sample. For this reason, it cannot be determined whether the larvae, which emerged on the second exposure to root excretion, were from eggs which had newly matured, or whether they would have been liberated in any case had the first root excretion been more potent.

The main conclusions which can be drawn from the "rehatching" of the treated cysts are (a) that the contents of those cysts which produced no larvae during their first exposure to root excretion were killed by the solutions in which they were immersed. This was demonstrated by the fact that, except in only two cases, all the cysts which previously produced no

Table VII.

Relative larval emergences from treated cysts after "rehatch".

Silver Compound	Concn. of solns.		Relative larval emergences (% of control)			
	%	Mgm. Silver p.litre	1 day	1 week	3 weeks	6 weeks
Prot.	0.05	40	1.6	0	3.8	0
	0.01	8	46.8	46.9	19.2	31.7
	0.005	4	82.3	65.6	7.7	20.09
	0.001	0.8	538.7	228.1	242.3	129.0
Acet.	0.05	323.5	0	0	0	0
	0.01	64.7	2.4	0	0	0
	0.005	32.35	85.5	0	0	0
	0.001	6.47	58.8	3.1	0	9.76
Benz.	0.05	292	0	0	0	0
	0.01	58.4	1.6	0	0	0
	0.005	29.2	235.5	0	11.5	2.96
	0.001	5.84	204.8	353.1	92.3	116.1
Lact.	0.05	274	1.6	0	0	0
	0.01	54.8	2.4	0	0	0
	0.005	27.4	796	28.1	7.7	1.01
	0.001	5.48	346	15.6	92.3	63.77
Control[x] (water)	-	-	100 (124)	100 (32)	100 (26)	100 (1383)

[x] Actual number of larvae shown in brackets.

larvae remained unproductive on rehatching. In these instances, 1 and 2 larvae were observed being 0.03% and 0.07% of the total output of the controls respectively, (Table VIII.). The action of the solutions was thus not merely to delay the hatching of the eggs but to kill them. (b) The stimulative action produced by certain concentrations of the silver compounds on the cyst contents was still apparent when the cysts were "rehatched".

Comparatively few larvae were liberated in the second emergence and, for this reason, the relative differences are rather exaggerated and thus inaccurate. The numbers of larvae liberated, however, tend to be in the same proportion as in the first "hatch" and, when the combined totals are calculated, the final relative emergences (Table VIII.) differ only very slightly, in most instances, from those obtained after the first part of the experiment (Table VI.). In some cases they are identical. The figures representing the first "hatch" therefore provide a reliable indication of the effect of the different solutions on the contents of H. schachtii cysts.

It may be of interest to note that, on examination of the total numbers of larvae (Table VIII.) which emerged from the cysts previously immersed in water for various lengths of time, a steady increase is evident, corresponding to increase in time of storage. It would thus appear that water itself

Table VIII.

Combined relative emergences for first and second "hatches".

Silver Compound	Concn. of Solns.		Relative larval emergences (% of control)			
	%	Mgm. Silver p.litre	1 day	1 week	3 weeks	6 weeks
Prot.	0.05	40	4.51	0.09	0.03	1.55
	0.01	8	64.12	46.95	19.26	38.91
	0.005	4	118.9	98.7	86.76	27.95
	0.001	0.8	302.1	191.7	108.4	112.7
Acet.	0.05	323.5	0	0	0	0.03
	0.01	64.7	0.76	0	0	0.03
	0.005	32.35	33.28	0.46	0.03	0.1
	0.001	6.47	66.07	5.91	8.11	11.01
Benz.	0.05	292	0	0	0	0
	0.01	58.4	1.18	0	0	0.13
	0.005	29.2	112.4	13.33	1.72	3.73
	0.001	5.84	200.7	178.8	119.2	105.7
Lact.	0.05	274	0.07	0	0	0
	0.01	54.8	3.5	1.83	0	0.08
	0.005	27.4	209.1	35.55	4.98	1.27
	0.001	5.48	187.4	53.64	54.76	50.87
Control[x] (water)	-	-	100 (2882)	100 (3451)	100 (3552)	100 (3862)

[x] Actual number of larvae shown in brackets.

has a stimulative effect on the eggs contained in eelworm cysts.

Silver proteinate was employed alone in further experiments on the pre-treatment of potato eelworm cysts taken from a sample which had been extracted from an infested field two years previously. The concentrations of the solutions used ranged from 0.1% to 0.001% and the times of storage from 12 hours to 3 weeks. Rehatching of these cysts was carried out after several months' storage, and the combined relative larval emergences are shown on Table IX.

The numbers of larvae which were liberated from these cysts were very much smaller than those observed in the previous experiment. The principal reason for this is that, although cysts may remain potentially infective for many years, this power gradually diminishes probably as a result of the breakdown of part of the contents. It may be on account of this also, that the critical toxic concentrations after various periods of immersion of these cysts in the solutions are lower than those found using newer cysts. Thus the silver contents of the solutions which control liberation of larvae from these older cysts after storage for 1 day, 1 week and 3 weeks were respectively about 35, 25 and 15 mgm.per litre, while the corresponding figures obtained in the previous experiment with newer cysts were in the region of 65, 40 and 35 mgm.per litre.

Table IX.

Relative larval emergences from cysts stored in solutions of silver proteinate.

Concn. of solns.		Relative larval emergences (% of control)					
%	Mgm. silver per litre.	12 hrs.	1 day	3 days	1 week	2 wks.	3 wks.
0.1	80	0	0	0	0	0	0
0.075	60	0	0	0	0	0	0
0.05	40	5.66	0	0	0	0	0
0.025	20	17.23	2.74	0.24	0.31	0	0
0.01	8	82.79	93.37	60.67	2.99	10.34	0.21
0.005	4	121.3	108.9	46.77	19.21	12.34	2.75
0.001	0.8	199.3	123.5	162.9	57.48	80.94	71.14
Control[x] (water)	-	100 (389)	100 (438)	100 (417)	100 (635)	100 (551)	100 (946)

[x] Actual number of larvae shown in brackets.

Both experiments indicated, however, that there was a gradual decrease in the numbers of larvae set free, proportional to the increase in the silver content of the solutions.

Increase in time of immersion of cysts in the silver proteinate solutions tended, in this experiment also, to decrease the output of larvae and once more the stimulative effect of the 0.005% and 0.001% solutions was shown, although

here it was most clearly observed after 12 hours. There was also a suggestion that with longer times of immersion of the cysts in water an increase in emergence is produced.

That the silver solutions have a speedy action on cyst contents is evidenced by the fact that, after only 12 hours' contact with 0.1% or 0.075% silver proteinate, cysts failed to show any sign of larval liberation. It was further found, using cysts from the same sample, that after storage for 6 hours in 0.1% silver proteinate no "hatch" was given, and treatment for 1 hour in the same concentration resulted in a reduction in larval output to 17% of the control.

It was shown that cysts of the potato strain of H. schachtii, whether recently formed or not, set free no larvae after 24 hours' treatment with 0.1% silver proteinate. (Actually from over 1200 cysts treated in this way, transferred to potato root excretion and examined, only 2 larvae were observed). Even after storing the cysts and "rehatching" them after long periods, no emergence took place, which gives a clear indication that the contents had been killed and that not merely a delay in hatching had been effected.

In order to obtain more information on this point, some relatively newly formed cysts treated in the above manner were crushed in a drop of water on a microscope slide. At the same time cysts which had been kept in water for the same

period were similarly examined.

No apparent difference was noticed in the egg cases from the differently treated cysts, but embryonic larvae contained in the eggs from cysts kept in the proteinate appeared to be more granular, although this feature could not be taken as diagnostic of death. Embryonic larvae, which had been forced out of the eggs in the process of crushing and lay with the body in the characteristic angled position appeared in much the same condition in both cases, but in many instances those from cysts treated with proteinate gave the impression of being darker at the head end than those from the control cysts.

Differences were, however, seen in the more mature larvae which had just escaped from the eggs. Although some from the control cysts were obviously dead, the majority had every appearance of being alive, the head end of the body being quite hyaline, and indeed some were seen to exhibit the normal wriggling motion. Mature larvae from the cysts treated with silver proteinate, on the other hand, all showed signs of being dead, since that part of the head end which is clear in the living larva had become granular and darker in colour, the whole larva assuming an almost uniform density.

On the basis of this evidence, it is considered that when no larvae are set free from cysts which have been treated with silver proteinate solutions, the contents of these cysts

have been killed. It is assumed also that when a much reduced "hatch" is observed, part of the cyst contents has been destroyed. It may be deduced that the other silver compounds act in the same fashion.

(b) Treatment of cysts in presence of root excretion.

The results of the experiments in which potato eelworm cysts were stored in silver proteinate solutions containing root excretion are shown on Table X. The cysts used had been extracted from infested soil only a few months before, and many of them, therefore, had been formed on the previous season's potato crop. Cysts from a sample which had been extracted two years previously were also used in a similar experiment, and the numbers of emerging larvae were proportionally smaller, but otherwise the results of both experiments were essentially the same.

With solutions of 20 mgm. or more silver per litre, either in water or in root excretion, for all practical purposes complete control of larval emergence was effected. Solutions, both in water and root excretion, which possess 4 and 8 mgm.silver per litre were not potent enough to destroy the cyst contents after 3 weeks, but it can be observed that more larvae were set free from the cysts which had been stored in the root excretion-proteinate solutions, indicating, as in experiments with larvae, a limiting of the effect of the silver

Table X.

Larval emergences from cysts treated with silver proteinate in presence of potato root excretion.

Concn.of Solns.		Larval emergences	
%	Mgm.silver per litre	21 days in original soln.	Further 42 days in root excn.
0.1 in Excn.	80	0	0
0.1 " Water,	80	0	0
0.05 " Excn.	40	1 (dead)	2
0.05 " Water,	40	0	0
0.025 " Excn.	20	0	0
0.025 " Water,	20	0	0
0.01 " Excn.	8	2 (dead)	114
0.01 " Water,	8	1 (dead)	29
0.005 " Excn.	4	16 (dead)	164
0.005 " Water,	4	3 (dead)	66
0.001 " Excn.	0.8	608 (598 dead)	1798
0.001 " Water,	0.8	6 (4 dead)	3834
Excn. 90%,	-	4015	3320
Excn.100%,	-	4699	2671
Water,	-	13	5990

by the root excretion. Further, during the action of these solutions, there was an attempt by the root excretion to

induce larval emergence. This effect is more clearly seen with 0.001% proteinate solution in root excretion, where 608 larvae were liberated as opposed to 6 larvae in the corresponding solution in water. In these liquids, however, the larvae which were set free in a living condition by the root excretion were immediately acted on by the silver and killed. Consequently after 21 days, of the larvae which emerged in the 0.001% proteinate-root excretion solution, 598 out of 608 were dead. Cysts stored in root excretion of the corresponding strength (90%), i.e. stored in a similar solution with no silver proteinate present, liberated over the same period 4015 larvae.

When, after 21 days, the cysts in the 0.001% proteinate solutions had practically ceased to produce more larvae, they were transferred to fresh root excretion and the resulting "hatches" were considerably smaller than those of the controls. The "hatch" from the cysts treated with the root excretion-proteinate solution, however, was very much smaller than that from the cysts in the corresponding water solution. The reason for this is twofold. Firstly, in the former case, a considerable number of larvae had already been liberated by the root excretion in the original solution, whereas the "hatch" in the latter case had been negligible. Secondly, as these larvae were set free by the root excretion, the silver in the solution had a better chance to penetrate the cysts and exert its toxic

influence on all their contents.

It will be noted from Table X. that the numbers of larvae, hatched from cysts stored in 90% root excretion, was smaller than that obtained from cysts immersed for a similar period in undiluted root excretion. When both series of cysts were transferred to fresh undiluted excretion from the same stock and observed over the same length of time until emergence had practically ceased, the former series this time liberated more larvae, making the total number set free in each case almost the same.

The toxic effect of the various concentrations of silver proteinate appears to be greater on the cysts in this experiment than on cysts from the same sample in the previous pre-treatment, whose results are noted on Table VIII. In the latter experiment, however, about one thousand cysts were treated with 2 cc.of the solutions, whereas in the experiment described above each set of 50 cysts was treated separately with this volume of the proteinate solution. There is an indication, therefore, that the toxic influence of silver proteinate is slightly less upon a large number of cysts than upon a small number. This is most probably the result of a quantitative effect on the part of the silver, but may conceivably be due to a mass effect of the cysts, comparable to that exerted by many other animal organisms.

(c) Treatment of cysts in samples of infested soil.

It will be remembered that this section of the investigation was carried out in three parts. The first was actually a control experiment in which cysts, similar to those used in the other experiments, were treated in watch-glasses with the proteinate solutions, before being transferred to root excretion. It was essentially an in vitro pre-treatment experiment, similar to those conducted previously, and yielded results (Table XI.) comparable to those shown on Table VI.

Table XI.

Relative larval emergences from cysts treated with silver proteinate in the soil and in vitro.

Concn.of solns.		Relative larval emergences (% of control)							
	Mgm.	1 day		1 week		3 weeks		6 weeks	
%	Silver p.litre	In Soil	In Vitro	In Soil	In Vitro	In Soil	In Vitro	In Soil	In Vitro
1	800	0.35	0	4.4	0	3.8	0	63.4	0
0.1	80	70.0	0	16.6	0.05	24.07	0	62.4	0.02
0.01	8	121.1	120.0	80.5	26.3	54.6	51.2	70.6	22.1
Control[x] (water)	-	100 (2851)	100 (4488)	100 (2137)	100 (4285)	100 (3161)	100 (2706)	100 (5376)	100 (6162)

[x] Actual number of larvae shown in brackets.

The second part involved the application of the

solutions to infested soil from which samples of cysts were periodically extracted, and their relative larval emergences observed and recorded also on Table XI.

The differences between the critical concentrations of silver proteinate necessary to inhibit liberation of larvae from cysts treated in soil and those treated in vitro are clearly manifest by a comparison of the corresponding emergences on Table XI. A solution of 1% proteinate was not quite sufficient to provide complete control of the parasite in the soil, and weaker solutions were correspondingly less effective. Increase in time of contact between the cysts and the applied solutions up to at least 3 weeks caused a general decrease in larval emergence, and a slight stimulation was given by the 0.01% solution after 1 day.

The cysts extracted from the soil 6 weeks after the solutions had been applied all showed a greatly increased output of larvae. The reason for this was that since samples of soil were extracted from time to time the levels from which the cysts were taken became progressively lower as more of the surface soil was removed. Thus after 6 weeks, the cysts were taken from 2-3 inches below the surface, where they had evidently not been in sufficient contact with the applied solutions. That the increased "hatches" were not caused by the effect of the substance having worn off, or that it had merely delayed the

emergence, is demonstrated by the complete and continued absence of hatching from cysts kept for a similar period in 1% and 0.1% proteinate solutions. It is also shown by the fact that those treated cysts, extracted after 1 day, 1 week and 3 weeks, which liberated only small numbers of larvae, showed no tendency to increase their output towards the end of their period of hatching. Silver proteinate, therefore, loses much of its potential effect on the contents of eelworm cysts, after penetrating the soil to a depth of 2-3 inches.

The third part of the experiment consisted of pre-treating cysts, similar to those used in the other two parts, with the filtrates obtained after the different solutions had percolated through the soils. The numbers of larvae set free by these cysts were variable and could not be correlated with the concentrations of the corresponding proteinate solutions, but they showed no sign of decreasing with time of immersion of the cysts in the filtrates. Even from cysts stored in the filtrate of the 1% solution, there was no reduction in the output of larvae, but rather on the contrary, a tendency towards an increased "hatch" was noted.

It may thus be stated that a 1% solution of silver proteinate, applied to eelworm infested soil in the amount required to saturate the soil, provided almost complete control of the parasite only in the top 2-3 inch layer of soil. Lower

concentrations gave smaller degrees of control, but again their main effect was confined to the surface layers. Before any of these solutions had penetrated to a depth of about 6 inches (the depth of soil in the funnel), all their toxic potentialities were dissipated.

When the last of these cyst samples had been taken, each of the soils was mixed thoroughly and further samples of cysts extracted. The respective larval emergences from the cysts from the 1%, 0.1% and 0.01% treatments were 39%, 82.7% and 95.8% of the control. When potato tubers were planted in these soils, the average infections found in the roots were respectively 21%, 121% and 134% of the control. Thus only the 1% proteinate solution provided a reasonable measure of control over the whole volume of the soil.

Discussion.

From these results it will be observed that the organic silver compounds used, even at very low concentrations, have a strong toxic influence on the contents of potato eelworm cysts. Variations in the toxicity of different dilutions of the compounds were measured by the relative numbers of larvae liberated by the treated cysts on exposure to potato root excretion.

That the action of the substances under investigation

was toxic was shown by the fact that when treated cysts, from which no larvae were set free under the influence of root excretion, were "rehatched", even after several months, inhibition of emergence was still maintained. Further, on examination of the contents of such cysts, signs indicative of death were noted particularly in the mature larvae. Incidentally, it may be possible on the basis of such an examination to determine the result of previous treatments with other compounds on the cysts, without resorting to the usual lengthy process of "hatching" with root excretion. Thus the silver compounds do not merely effect a delay in larval emergence such as is given by certain concentrations of calcium chloroacetate (33)(120). Even solutions containing an intermediate quantity of silver, which do not provide complete control of hatching, do kill a proportion of the cyst contents and have more than a temporary effect on them.

The toxic influence which these solutions exert on the eggs and larvae within the cysts was found to be dependent on the silver contents of the solutions, and in spite of certain variations, this was maintained for all the compounds used with the exception of silver nucleinate.

Silver nucleinate did not act on the contents of the cysts in a manner comparable to any of the other substances used, for although this salt contains 20% silver, a 0.1% solution did not completely control larval emergence, even after 6 weeks'

contact with the cysts. It will be remembered that this compound behaves towards larvae in a fashion which is peculiar and unlike that of the other substances, and the silver again appears to be very closely bound in the molecule, decreasing the toxicity of the compound as a whole.

Silver proteinate was employed in the experiments where it was possible to use only one substance, since the proteinate was as effective as the other compounds at corresponding silver concentrations, and also since it gave no turbidity with sodium chloride at strengths corresponding to those of silver acetate, benzoate and lactate which did give a turbidity. Because of this, the proteinate seemed to be much more suitable for application to the soil than the other substances. Furthermore, the proteinate was used by Hovy (44), who claimed that it had given the most satisfactory results.

The toxic effect of the silver solutions was apparent even after 1 day, when a concentration of only 65 mgm. of the element per litre, i.e., 65 parts per 1,000,000 was sufficient to give complete control. The effect increased with the length of the period of action of the solutions on the cysts, so that after 3 weeks the critical silver concentration was in the region of 40 mgm. per litre.

It was also shown that the action of silver was greater on older cysts than on those which had been more recently

extracted from the soil. That older cysts are not so productive in larvae as newer ones was established by Franklin (24) for cysts from the Yorkshire and Lincolnshire areas, and was also shown by the writer, in experiments described in a later paper with cysts from the same field and from the same samples as those used in the investigations with silver compounds. Many of the eggs in older cysts appear to be destroyed and parasitised by fungi and thus the toxic solution has a greater chance to penetrate the whole volume of the cyst and attack all parts of the cyst contents. This is probably the reason why lower concentrations of silver, e.g. about 15 mgm.per litre, can after 3 weeks fully inhibit larval emergence from cysts 2-3 years old.

There are some factors which decrease the influence of the silver solutions on the cyst contents. When potato root excretion is present during the action, although it induces some of the larvae to emerge into the toxic silver solution, it also causes a slight limitation in the effect of this solution on the cyst contents, probably because some of the silver becomes adsorbed on organic particles in the root excretion. It will be recalled that a similar factor was encountered in experiments with the larvae of the parasite. If the solution with root excretion present has a silver content of about 0.8 mgm.per litre, a type of "ideal solution" is formed, according to one method of controlling the parasite. In this mixture, the root

excretion causes many of the larvae to emerge and these are killed within a few hours by the action of the silver in the solution. During this time, the silver itself penetrates the cyst and exerts a lethal effect on a greater part of the contents than would otherwise have been the case, had not many of the larvae already escaped and allowed the solution a more intimate contact with the closely packed eggs, in the same way as happens with older cysts. Complete control was, however, not obtained with this solution, since some 25% of the cyst contents remained undamaged and viable.

A quantitative effect was also noted on the part of the silver proteinate, i.e. it exerted a slightly stronger toxic influence on a small number of cysts than upon a large number.

The factor which has the most serious limiting effect upon the action of solutions of silver compounds on the cyst contents of H. schachtii is the soil. When a solution of a concentration which will completely prevent larval emergence from cysts in vitro was applied to eelworm-infested soil, much of its effect was lost even in the surface layers. A solution of 1% silver proteinate, containing 800 mgm. silver per litre, which did provide practically complete control in the top 2-3 inches of soil, had a much reduced influence below this level, and before penetrating to a depth of 6 inches, even this effect had gone. The silver appears to have been bound in the upper layers of the soil

and was probably adsorbed on the organic matter, although the soil used was of an extremely sandy nature. It was as a solution of this very problem of adsorption that Hovy (44) suggested the use of an organic silver compound.

Dilute solutions of silver compounds containing about 1 mgm. of the element per litre had a stimulatory effect on the contents of potato eelworm cysts immersed in them for periods of about 24 hours. This effect was not merely temporary but was maintained even when the cysts were "rehatched" after several months. A similar phenomenon was observed in connection with the action of solutions of certain silver compounds on the free larvae of the nematode. In this case it was suggested that the hyper-activity of the worms was analogous to the "flight movements" described by Baunacke (1).

When dilute silver solutions act on the cyst contents, they must effect some change in the eggs. That they do not act like potato root excretion and cause larvae to emerge directly when cysts are kept in the solutions, was seen by observing the small numbers of larvae in these solutions, comparable to the numbers which emerged in water. Pre-treatment of cysts with certain dilutions of calcium chloroacetate has also been found (33)(120) to cause the liberation of an increased number of larvae when the cysts were transferred to root excretion. It was found by Molz (66) and Smedley (89), that weak bleaching powder

solutions caused stimulation of hatching, not only from cysts placed in root excretion but also from cysts kept in the original solutions. Molz attributes the increased rate of hatching to a physiological stimulus, and believes that it is due to the property of chloride of lime of liberating free oxygen, and to the alkaline nature of the compound. Smedley, however, is of the opinion that the stimulating power of the hypochlorites is mainly the result of their peculiar action on proteins, and that hypochlorite solutions which contain calcium attack the egg membranes in the eelworm cysts, inducing the eggs to hatch without the presence of root excretion.

The stimulative power of the silver solutions may also be due to their effect on the proteins, for in cases where silver exerts a toxic influence on protoplasm, the principal reaction, according to Seifriz and Uraguchi (86), probably takes place between the silver and the protein.

The net result of the brief action of the dilute silver solutions is to prepare the eggs so that the root excretion can exert its maximum effect on the cyst contents and thus set free many more larvae than would be the case had the cysts been pre-treated with tap water. This implies that very many more of the eggs in a cyst are ready for hatching, i.e., mature, than can be accounted for by the number of larvae which normally emerge when the cyst is placed in contact with root excretion and

kept even until no more larvae are liberated. Hence it may be deduced that in the cysts used - apart from any immature eggs which there may or may not have been - there were mature eggs which hatched directly under the influence of root excretion, and also mature eggs which hatched only indirectly, after a brief pre-treatment with a dilute solution of a silver compound. This is in keeping with the known fact that all the eggs contained in potato eelworm cysts will not liberate their larvae during the first year in which the cysts are in contact with the excretion from potato roots. Many of the eggs remain unhatched in the cysts for some undiscovered reason, and serve to carry over the potential infection for at the very least two years. This happens under natural conditions, and in laboratory experiments, as far as is known, no potato root excretion has yet been produced which will regularly cause all the eggs in newly formed cysts to hatch during the first period of contact between the cysts and the root excretion, although Triffitt (112) indicates that all eggs in overwintered cysts are capable of hatching under favourable conditions in the next season after their formation.

The stimulative action of the silver solutions may be, on the other hand, to cause immature eggs to mature before the root excretion begins to take effect. If this is so, maturation must occur very quickly so that the eggs are immediately ready to hatch, for not only did larval emergence from stimulated and

control cysts commence and finish practically simultaneously, but the graphs of their progress reached their maxima also at the same time. In view of this, it is hardly likely that the effect of the silver solutions was to bring about maturation of the eggs. It may be concluded, however, that they act on the eggs in such a way that subsequent treatment with potato root excretion induces an abnormal number of them to hatch, and that although many more larvae are produced than from control cysts, the periods over which they emerge are practically the same.

Evidence that there is an increase in the output of larvae from cysts stored for increasing lengths of time in tap water was also observed. This is not stimulation of the type shown by silver compounds but is probably the result of absorption of water which encourages and influences the development and maturation of eggs within the cysts.

B. Experiments with Cysts (continued)

(2) Pot Experiments.

It may be found, from in vitro experiments, that certain substances are extremely toxic to both larval and cystic stages of the potato root eelworm. The application of these substances to the soil introduces a multitude of complex and variable factors which, in the majority of cases, decrease the effect of the compound on the nematode. Evidence of this for silver proteinate could be seen in the experiments previously described where this substance was applied to the soil and its effect upon the cysts contained in the soil determined. The conclusions thus obtained, however, were not available when most of the following pot experiments were undertaken.

Materials and Methods.

Silver proteinate was used in all the pot experiments for reasons which have been stated before. One of these reasons, the fact that silver proteinate does not form a turbidity with solutions of sodium chloride, assumes special importance when the compound is applied to the soil, because of the presence of common salt in soils, especially those of a light nature situated near the sea. It is often such areas which are given over to the cultivation of potatoes, and also such soil which appears to be

most suitable for the propagation of the eelworm.

The soil used was the same as that from which some of the cysts employed in the in vitro experiments had been extracted, and had been brought from the Warren field, Jameston Farm, Maidens, Ayrshire, a field which was known to have a very high cyst population. The soil was of a light and sandy nature, having a moisture content of about 10%, and was very suitable for the cultivation of the early potato crop.

Experimental Methods.

Experiments to investigate the effect of organically combined silver, represented in silver proteinate, in controlling potato eelworm in the soil, were performed by (a) an investigation of the effect of the compound on the crop and on larval invasion of the rootlets, (b) the determination of the most efficient rate of application of the compound, and (c) an investigation of the action of the compound on the potato plant itself.

(a) Effect of silver proteinate on the crop and on larval invasion of rootlets.

Different concentrations of the proteinate were applied to the infested soil, which was contained in porous pots of 10 inch diameter, 100 cc. of the requisite solution being poured on the soil by means of a pipette and each treatment

being replicated six times. The range covered by the concentrations was 0.002% to 2%, but taking into account the moisture content of the soil, the corresponding final concentrations of the compound in the soil varied from about 0.00025% to 0.25%. The amounts of silver proteinate applied per pot - 0.002 gm. to 2 gm. - correspond by calculation to field treatments of from 0.5 lb. per acre to 5 cwt. per acre.

One well-sprouted, weighed, Epicure seed tuber - the variety which is ubiquitous in the early potato growing districts of Ayrshire - was planted in each pot. The tubers were chosen so as to be as uniform as possible in bulk and length of sprout, and before planting they were washed to dispose of any adhering cysts. The plants were observed carefully during their period of growth, and when they had died down, the yields of tubers were weighed. Samples of the differently treated soils were also taken at the conclusion of the experiment, cysts from each sample extracted and the relative larval emergences counted.

At intervals during the life of the plants, estimations were made of the amount of infection in the roots after the different treatments. The method employed was that outlined by Gemmell (31), and consisted in taking a few representative samples of the roots from each plant. These were washed and transferred to 90% alcohol, after which lengths of 0.5 inch

were excised from the finer rootlets, care being taken to include the rootcap. These rootlets were thus all of approximately the same age and had had the same length of exposure to infection. They were then stained in a 2% solution of iodine in alcohol for about 90 minutes (which was found to be a more satisfactory period than that indicated by Gemmell), destained for a short time in 90% alcohol until the roots were colourless, cleared in clove oil and finally equal numbers of the rootlets from each treatment were mounted in a drop of clove oil and examined by means of a binocular microscope. In this way the larvae, which are more retentive of the stain than the rootlets, can be easily seen and counted and the percentage infection of a given plant can be calculated on the ratio of the numbers of infected rootlets observed to the total number examined.

This experiment was repeated using silver proteinate at the same rates as before, but applying it as a powder mixed with very fine sand in order to obtain a more uniform spread. The compound was then stirred or "cultivated" into the top 3-4 inches of the soil.

(b) Optimal rate of application of silver proteinate.

The methods used in this experiment were similar to those in the previous tests, but applications of the compound in powder form ranged from 2 gm.per pot to 16 gm.per pot, i.e., from 5 cwt. to 40 cwt. per acre, the soil having been

treated with a complete artificial fertiliser at the relative rate of 10 cwt. per acre. In this experiment the soil for the four replicates of each treatment was thoroughly mixed with the proteinate before potting, so that the compound should have an even distribution throughout the soil. The control plants were grown in infested soil which had received an equal amount of fertiliser, but was otherwise untreated. Periodic root examination was carried out, and again, after the crop had been weighed, cyst samples were extracted and "hatched".

An experiment, similar to this in all other respects, was performed at a different time of year.

(c) Effect of silver proteinate on the potato plant.

The ideal chemical for the control of a parasite should obviously exert all its toxic powers on the invader and leave the host undamaged. Very few substances, if any at all, attain this high standard, and since silver compounds were unlikely to prove exceptional in this respect, the action of silver proteinate was determined by experiment.

Eelworm-free sand with a moisture content of 4%, having received an application of complete artifical festiliser equivalent to 15 cwt. per acre, was treated with the requisite amount of silver proteinate which, in the three replicates of each treatment, was thoroughly mixed into the sand before potting, there being 20 lb. sand in each pot. The substance

was applied at the rates of from 0.2 gm. to 20 gm. per pot, which correspond to field treatments of from 0.5 cwt. to 50 cwt. per acre, and which in the pots gave concentrations of proteinate solutions covering the range 0.06% to 6%. One sprouted Epicure tuber was planted in each pot and the resulting plants examined from time to time, the numbers of haulms and heights of the plants being noted. The yield of tubers in each pot was weighed when the plants had died down.

Later, another series of plants was grown in the same sand, after suitable treatment with complete fertiliser, to determine the residual effect of the proteinate on a second crop of potatoes.

Experimental Results.

(a) Effect of silver proteinate on the crop and on larval invasion of the rootlets.

The first experiment, in which the proteinate was applied in solution, was conducted in winter and spring and very slow initial growth was made by the plants on account of the cold conditions although the pots were kept in a heated greenhouse. The first appearance of infection in the roots was observed about two months after planting and about 14 days after all the plants had emerged above the soil. The results of this test are summarised on Table XII.

Table XII.

Effect of application of silver proteinate solutions on the larval invasion of potato rootlets and on the crop produced.

Relative Application per acre.	Concn.of Soln.in Soil (%)	Average Rootlet Infection (%)	Average Number of larvae p.Rootlet	Increase in Crop from Seed planted (%)
5 cwt.	0.25	62	1.7	56
2.5 "	0.125	69	2.5	36
56 lb.	0.025	71	3.0	23
28 "	0.0125	78	3.5	24
5.6 "	0.0025	70	3.0	15
2.8 "	0.00125	72	3.4	3
0.56 "	0.00025	70	3.2	19
Control (untreated)	-	75	3.4	27

No difference was observed in either the rate of emergence or the rate of dying off of the plants of the different treatments. Counts of the larvae[x] in root samples were made regularly from the first appearance of infection in the control pots, until that stage in the life of the plants, when no more rootlets were produced. The maximum infection at any one time in the control plants was 96%, and that in the

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x The larval counts in this experiment were made by Dr.A.R. Gemmell to whom thanks are due.

plants of the 5 cwt. treatment 76%. Invasion of the rootlets by H. schachtii larvae took place in all treatments on a relatively heavy scale until the cessation of rootlet formation, but a decrease in average infection was noticeable after the heaviest application of proteinate, which also provided a definite decrease in the average number of larvae per rootlet, while smaller decreases were given by 2.5 cwt. per acre.

Since in pot experiments the yields of tubers produced by the plants are generally smaller than in the field, thus making relative differences more difficult to estimate, the percentage increases in weight of crop over the weight of seed planted were noted. Again only the heaviest treatment provided an increase significantly larger than the control.

No consistent differences were observed in the larval emergences from cysts extracted from the variously treated soils.

The results of a similar experiment, carried out in summer with silver proteinate applied as a powder, confirmed the previous findings. Although in plants from the heaviest treatment an increase in the yield of tubers, a lowering of the average infection and a substantial decrease in the number of larvae per rootlet were found, the infections were not so great as in the first experiment, the highest estimation being 68%. The rate of larval invasion of the rootlets appeared to

reach a maximum and then began to decrease in a manner similar to that generally shown by larval emergences from cysts which have been placed in potato root excretion.

Significant differences were again absent in the "hatches" from cysts taken from the treated soils.

(b) Optimal rate of application of silver proteinate.

Detailed results of experiments, carried out in winter and summer, in which eelworm-infested soil was treated with the heavier relative applications of silver proteinate of from 5 cwt. to 40 cwt. per acre, are shown on Table XIII.

The plants all emerged above the soil at about the same time, and showed no differences in heights, but those growing in soil treated with 30 cwt. and 40 cwt. per acre of the proteinate had a darker green appearance. It was noted that the 40 cwt. treatment caused the plants to die off most quickly, while those in soil to which 10 cwt. per acre had been applied were last to die off, the next in order being the plants in the 15 cwt. and 5 cwt. treatments.

The average larval invasion of the rootlets was lower in the winter experiment than that in summer, most probably on account of the temperature or perhaps because of a dormancy period of the cysts, but even so, the summer invasion was not very large, the highest estimation in the untreated plants being 75%. It was observed again in these experiments

Table XIII.

Effect of application of silver proteinate on the larval invasion of potato rootlets and on the crop produced.

Relative Applicn. per acre	Concn. of soln. in soil (%)	Winter			Summer		
		Average infectn. (%)	Average number of larvae per rootlet	Weight of crop (% of control)	Average Infectn. (%)	Average number of larvae per rootlet	Weight of crop (% of control)
40 cwt.	2.0	0	0	73.9	2.5	0.03	93.5
30 "	1.5	0	0	69.6	5.8	0.15	103
20 "	1.0	0	0	95.7	9.0	0.18	110
15 "	0.75	3.4	0.04	104.3	7.0	0.07	121
10 "	0.5	7.8	0.05	113.0	17.4	0.28	121
5 "	0.25	5.9	0.06	95.7	23.8	0.45	95.2
Control (untreated)	0	20.3	0.33	100	33.3	0.68	100

that the rate of penetration of potato rootlets by larvae was not constant, but decreased sharply after a maximum rate had been attained.

All the treatments provided some degree of control of larval invasion, the numbers of rootlets infected and numbers of larvae in these rootlets varying inversely with the amounts of silver proteinate applied. No sign of infection was observed after treatment of the soil with 20 cwt. per acre in the winter experiment, but in summer twice this application

was not quite sufficient to control the parasite completely. The average infection after this latter treatment was 2.5%, the maximum figure observed being 6%.

When the relative yields of tubers produced by the plants are examined, however, it can be seen that while certain applications of the compound have the effect of increasing the crop, evidently by a reduction in the numbers of larvae invading the roots, these increases are not maintained at higher rates of application and the most effective dose is in the region of 10-15 cwt. per acre. This treatment produced the maximum crop and although it left 17% infection in the rootlets, any further attempt to decrease larval penetration by increasing the amount of the compound applied, resulted in a decrease in crop. Above this critical point, where the concentration of the compound in the soil was between 0.5% and 0.75%, the harmful effect of the silver on the plant itself outweighed the benefit of its toxic effect on the parasite.

When cysts from the treated soils were "hatched", there was a tendency for those from the 20, 30 and 40 cwt. treatments to liberate fewer larvae than the control cysts. This decrease, however, was consistently obtained only from the cysts which had been in contact with the highest application of silver proteinate.

(c)

(c) Effect of silver proteinate on the potato plant.

The injurious effect which is exerted by the compound on the potato plant, when grown in a medium of pure sand in the absence of H. schachtii, can be seen from the data presented in Table XIV.

The plants in contact with the heaviest doses of silver proteinate were last to emerge and made the slowest growth. In appearance the plants fell into three groups corresponding to the respective concentrations of the compound. No difference was observed in the appearance of the untreated plants and those grown in presence of 0.06% and 0.6% silver proteinate. The three concentrations from 1.2% to 2.4% produced only slightly smaller leaves of a darker green colour, but the three heaviest applications caused the plants to have an extremely stunted appearance with small, shrivelled leaves of a very dark green.

The sum of the heights of all the haulms provides a satisfactory estimation of the size of the aerial parts of potato plants grown under similar conditions, and it was found that, in plants in contact with more than 1% silver proteinate, not only when they were fully grown but throughout their period of growth, there was a gradual decrease in size of the aerial parts corresponding to increase in concentration of the compound.

Table XIV.

Effect of silver proteinate on uninfected potato plants.

Relative application per acre	Concn. of soln.in sand (%)	Average height of plants (% of control)	Weight of crop (% of control)
50 cwt.	6	32.2	33.3
40 "	4.8	32.2	29.2
30 "	3.6	40	37.5
20 "	2.4	66.4	45.8
15 "	1.8	55	45.8
10 "	1.2	62.2	45.8
5 "	0.6	105	70.8
0.5 "	0.06	100	79.2
Control (untreated)	-	100	100

An examination of the weights of tubers produced also shows a steady decrease with increase in rate of application and even plants treated with 0.5 cwt. and 5 cwt. per acre yielded smaller crops than the control. The diminution in yield of tubers caused by the two lowest applications, however, was not observed when this part of the experiment was repeated.

Several months after the whole experiment had been concluded, a fresh crop of potatoes was grown in the original

sand, but no consistent differences were found in either the heights of the plants or the yields of tubers produced. Thus the deleterious effect of the silver was not apparent on a second crop, even although the relative application in the first instance had been as heavy as 50 cwt. per acre, and the compound must have been either taken up by the plants or, more probably, washed out of the sand.

Discussion.

From the results of the pot experiments, it can be seen that treatment of potato eelworm-infested soil with certain quantities of an organic silver compound, such as silver proteinate, does give a measure of control of the parasite. In a soil with a moisture content of about 10%, such as that used in the course of these tests, the minimum application to have any evident effect on the nematode, as observed by its invasion of potato rootlets, was 2.5 cwt. per acre. This dose when uniformly distributed throughout the soil gives a concentration of proteinate of between 0.1% and 0.2%, which is equivalent to a concentration of silver of 80 - 160 mgm. per litre.

The toxic effect of the compound on H. schachtii naturally becomes greater with corresponding increases in the amount of proteinate applied, but it was observed that treatment with even 40 cwt. per acre failed to control the eelworm

completely. The action of the substance does not directly result in benefit to the potato plant but rather indirectly by preventing the penetration of the rootlets by the larvae. The course of larval invasion was observed directly by staining root samples, and decreases in the numbers of infected rootlets and in the average numbers of larvae they contained corresponded very closely to the amounts of the compound applied.

The rate of invasion of the rootlets by the larvae, i.e. the rate of larval emergence from the cysts was only in the first experiment (Table XII.) maintained at a high level during the whole course of the production of new rootlets by the plant. In the other experiments, even those performed in summer, the rate of larval penetration rose quickly to a maximum (which was not so high as that shown in the first experiment) and then began to decrease until the infection of new rootlets was negligible. It is difficult to account for these different types of larval penetration of rootlets, but similar types of in vitro cyst "hatching" have been observed. In some cases larval emergence rises to a maximum and then falls off, the whole hatching period occupying only 30-40 days. This corresponds to the type of penetration rate found more often in the pot experiments and it is not easy to understand how under normal conditions a larval invasion of potato rootlets after this manner could set up a severe outbreak of potato

sickness. In the other mode of cyst "hatching", which has not been found so often by the writer in tests carried out in vitro, liberation of larvae is maintained over a period sometimes as long as 120 days and several maxima in the rate of emergence may be noted. Penetration of potato rootlets by larvae in the field probably takes place in a manner which corresponds to this latter type of larval emergence. Although the variations may be connected with the age of the cysts or differences in maturation of the eggs, much more work along these lines is necessary to determine the true cause.

No examples of increased rate of penetration of rootlets by the larvae resulting from stimulation in hatching of the cyst contents were observed in the examinations of stained root samples, probably because those applications of the compound which could have caused stimulation were in contact with the cysts for too long a period.

No permanent effect appeared to be left on the resting stages of the parasite in the soil by any of the treatments except by 40 cwt. per acre which alone provided a consistent decrease from the control in larval emergence from cysts extracted from the soil after the potato plants had died off.

The practical aspect of the attempts to control H. schachtii by means of organic silver compounds is limited, since when the concentration of the compounds in the soil

reaches a certain level, the harmful effect on the plant begins to play an important part. The presence of silver even in weak solutions does not appear to be of any beneficial value to the potato plant, but with solutions of silver proteinate up to about 0.75% the toxic action of the metal is greater on the parasite than on the host. Above this concentration, i.e. above a practical application of about 15 cwt. per acre, the plants begin to suffer more from the cure than from the disease. The treatment which is most efficient in keeping the incidence of larval invasion of the rootlets at a minimum and producing the maximum increase in yield of tubers was found to lie between 10 cwt. and 15 cwt. silver proteinate per acre. With this application it was possible to raise the weight of the crop by 21% and to lower the rootlet infection to about one third of the control.

Potato plants grown in sand treated with silver proteinate were affected to a greater extent by certain quantities of the compound than plants grown in presence of the same amounts in eelworm-infested soil. Thus an application of 40 cwt. per acre to the soil decreased the crop in two cases by 24% and 6.5%. In sand, however, there was a decrease of over 70% in crop and even a reduction to about the same degree in the aerial parts of the plants. This was probably due to at least two factors. (a) The presence of water in some form is

necessary before silver proteinate can act to any appreciable extent on either the eelworm or the potato plant. Thus, by reason of the lower moisture content of the sand, 40 cwt. per acre gives a concentration of 2% in the soil and 4.8% in the sand, while an application of only 17 cwt. per acre is necessary to form a 2% solution in the latter medium. (b) Although the soil used was of a very sandy nature it nevertheless contained a certain amount of organic matter. It is known that silver ions exhibit a strong tendency to be adsorbed on organic matter and there is evidence that a certain amount of silver proteinate becomes in some manner bound in the soil, with the result that its toxic action on both plant and nematode is greatly reduced.

Silver proteinate may have been more efficient in controlling H. schachtii in pot experiments if other methods of incorporating the substance in the soil had been employed, such as mixing it through the soil in the form of a solution instead of a powder, or applying it at a very heavy rate some time before planting the potatoes to allow its phytocidal properties to subside. The acid test, however, of a satisfactory chemical method of plant disease control, as indeed with many other things, is practicability. Treatment with silver proteinate showed this quality in the protection of the tobacco plant from H. marioni, but gave no sign of it when

used against the potato strain of H. schachtii. The application of such quantities of silver proteinate as 10 and 15 cwt. per acre is of course extremely uneconomical and therefore impracticable and moreover even at these heavy rates the compound does not give the plants complete protection from the nematode. Hovy (44) does not record the action of silver compounds on the latent stages of the tobacco eelworm and it must be remembered that in his hot-house experiments it was the larval stage of H. marioni which was affected, but he did obtain a practicable means of control by using 0.3 gm. per plant which, on the basis of the numbers of plants per acre, he reckoned to be equivalent to 3 metric pounds per acre. If instead, the application per acre is calculated on the weight of soil to a depth of 9 inches as has been done in the pot experiments previously described, 0.3 gms. per plant is equivalent to approximately 84 lb. per acre. However, neither this treatment nor any other which could have been used on a field scale provided satisfactory control of the potato root eelworm.

Conclusions.

Since 1859, when Heterodera schachtii was first discovered as a parasite of sugar-beet, strenuous efforts have been made to effect its eradication. Many methods of control have been devised including that which aims at the destruction of the cyst contents by chemical means. Many substances have been tested for this and some have given encouraging results, but none has warranted its employment in general agricultural practice as a control measure for "eelworm disease".

In an attempt to combat that strain of H. schachtii which attacks potato roots, organically combined silver was used. In the experiments which have been described this element exerted a very strong and relatively quick nematocidal action not only on the larvae but also on the contents of the resting cysts of the potato eelworm.

The relative activities of all the silver compounds used, with the exception of silver nucleinate, were dependent on the respective contents of the element, and in experiments performed in vitro with free larvae of the nematode, no difference was apparent in the action of organically and inorganically combined silver. The criterion of larval death mentioned by Baunacke (1) was used and found to be satisfactory as a basis for comparison of the relative toxicities of the different solutions.

In vitro experiments also showed that solutions with a silver content as low as 6 parts per hundred million exerted a toxic influence on potato eelworm larvae in water suspensions. These larvae are normally induced to hatch from the eggs and emerge from the cysts only under the action of potato root excretion and consequently this liquid is the natural medium in which the larvae are found. Root excretion appears to have a protective action on the larvae which are not so susceptible to the effect of silver when the excretion is present, the critical solution having a silver content in the region of 4 parts per ten million.

The limiting of the action of the silver by root excretion is also seen, although to a smaller extent, in experiments with cysts. It is believed to be due to the fact that in common with other heavy metals, according to Seifriz and Uraguchi (86), silver tends to be adsorbed on protoplasmic proteins. These occur in abundance in potato root excretion since this liquid, prepared in the manner previously described, as well as containing the actual excretion from the potato roots, possesses also minute protoplasmic particles which have been leached out of the soil. The same limiting phenomenon was witnessed although not to such a marked degree using mercury solutions, which were at the same time seen to be less effective in their toxic action on eelworm larvae, both in

water and in root excretion, than the corresponding solutions with equivalent silver contents. Thus the excretion from the potato roots, as well as causing the larvae to emerge from the resting cysts and guiding them to the potato rootlets, serves, by reason of the organic matter it contains, together with the soil solution to protect them to a certain extent from such toxic elements as silver and mercury, while the larvae are passing through their most vulnerable phase between the comparative safety of the cyst on one hand and the potato rootlet on the other.

Although a strict comparison is not possible it seems likely, from the evidence available, that larvae of the tobacco eelworm H. marioni are more susceptible to the toxic influence of silver compounds than those of the potato strain of H. schachtii. This may be one of the reasons why silver proteinate was used successfully to control the former nematode, while complete control of H. schachtii, using even stronger concentrations of the substance, was not obtained.

The critical silver concentrations of the solutions which are lethal to the cyst contents of the nematode are much greater than those of the solutions which effect the death of larvae, and appear to vary with the age of the cysts. Using a sample in which the most recently formed cysts were less than 1 year old, the critical solution for 1 day's immersion of the

cysts had a silver content of 65 mgm. per litre (65 parts per million) and when the cysts were treated for 3 weeks 40 mgm. per litre was the minimum silver content of the solution effecting complete inhibition of larval emergence. With 2-3 year old cysts, the necessary strengths are lower and 15 mgm. silver per litre is sufficient to kill the contents of these cysts after 3 weeks.

The reason for this appears to be that due to degeneration of part of the contents of the older cysts the toxic solutions can penetrate a greater portion of the egg mass than would otherwise be possible. The fact that larvae can be killed by silver solutions of a strength several hundred times lower than the lowest concentration which is lethal to the cyst contents, may be due to the protective power of the cyst wall which possibly adsorbs part of the element. On the other hand it may indicate a resistance to the toxic solutions on the part of the eggs and the embryonic larvae still within the egg cases.

Certain of the lower concentrations of silver compounds, before bringing about the death of the larvae, cause them to move more actively than untreated larvae. This hyperagility may be akin to the "flight movements" observed by Baunacke (1) with the beet strain of the same nematode. It may be a form of the same phenomenon produced by a different

agency. Silver solutions, weaker than those which kill the cyst contents, exert after a short period of contact with the cysts a stimulatory action upon them, causing many more larvae to emerge in potato root excretion than from untreated cysts. Other substances, such as calcium chloroacetate (33)(120) and calcium hypochlorite (66)(89), also have this property, but treatment with certain concentrations of the latter compound causes the cysts to liberate larvae without the presence of root excretion. Increases in time of storage of cysts in water was shown to produce increases in larval emergence from the cysts although the time necessary was longer and the increases obtained smaller than with the chemicals mentioned indicating that probably a different type of stimulation is concerned.

These various facts concerning stimulation of cyst contents and the production of increased "hatches" from cysts treated with certain chemicals lead to the conclusion that in H. schachtii cysts there are many more eggs which are mature i.e. ready to hatch, than can be accounted for by a normal larval emergence through the agency of root excretion alone.

Whether there are in the cysts layers of eggs to which the root excretion cannot penetrate or what the mechanism is which prevents apparently mature eggs from hatching is as yet unknown.

It is difficult to make true comparisons in toxicity

to H. schachtii between silver compounds and other substances. Silver proteinate, however, which contains only 8% silver appears to be more effective in bringing about the death of eelworm larvae, in the course of in vitro tests, than are similar concentrations of mercuric chloride and calcium chloroacetate. From recorded evidence (91) it also seems to be slightly more satisfactory for this purpose than phenyl isothiocyanate. The effect of silver salts with a higher silver content are even greater than that of silver proteinate and these facts place compounds of this element amongst the most toxic known to the larvae of the worm. In pot experiments with larvae their efficiency, as typified by that of silver proteinate, is not maintained to such a high degree. The action of this compound on cyst contents in vitro is much greater than the effect of calcium chloroacetate, 0.1% of which kills cyst contents within about 1 month (33) but is not so strong as that of phenyl isothiocyanate (91), 0.001% of which can kill cyst contents within 3 days.

Thus from in vitro experiments organic silver compounds with even a low silver content appear to have a much stronger toxic influence on the parasite than some of the substances which have been suggested as control agents, and probably the isothiocyanate group of compounds is among the few which are over all more effective.

When silver proteinate was applied to eelworm-infested soil in such a quantity as to saturate the soil, the effect of the silver on the cysts was found to be confined mainly to the top 2-3 inches of soil and before the solution had percolated to a depth of 6 inches, all its toxic action had been lost. Again when this compound was applied to pots of eelworm-infested soil in which potatoes were growing the theoretical beneficial results which should have been forthcoming were not obtained. A measure of control was given by an application of 10-15 cwt. per acre, but even this highly uneconomical dose failed to provide complete control and it was found that higher rates of application could not be tolerated by the potato plants.

Unsatisfactory incorporation in the soil undoubtedly hinders silver proteinate from exerting its maximum effect upon the cysts in infested soil but this problem is common to all methods of chemical control. With the silver compound there is also evidence that much of its influence is lost in the soil by other means and it is thought that in spite of Hovy's use of this substance to prevent adsorption of the silver on organic matter in the soil, this phenomenon does occur and is responsible for much of the loss in activity of silver proteinate.

It may thus be concluded that as far as the control

of the potato strain of H. schachtii is concerned, application of silver proteinate or any of the other silver compounds used in this investigation could not be undertaken with economical results. For these compounds at any rate it can be seen that between academic interest and practical importance there is a great gulf fixed, and for this reason laboratory investigations should precede practical soil treatments, if chemical control of diseases such as eelworm disease of potatoes is to be placed and maintained on a scientific basis.

Although practicable methods of control of Heterodera schachtii using silver compounds have proved abortive, much has been learned and several interesting facts established concerning the biology of the nematode. The failure of silver compounds to control the parasite is of course by no means unique, for in spite of all the time and money spent and all the chemicals tested, no satisfactory means of combat has been evolved. Many workers have been so impressed by this and so disappointed by the unscientific method of trying one substance after another that they have begun to lose faith in the immediate chemical control of the disease. That the problem is urgent is beyond doubt and with its solution in view many workers have carried out valuable investigations. That their efforts have so far failed to disclose a substance which could be readily utilised in general agricultural practice is no

reflexion on the ability of the investigators, but rather as Baunacke (1) has suggested because our knowledge of the parasite and its various reactions is as yet too incomplete for us to have learned the vital point of attack.

Chemical methods have until now been directed mainly against the cystic stage which although being the permanent seat and focus of infestation, and thus the easiest to attack, is nevertheless the most resistant phase of the worm. On the other hand most of the chemicals employed for control purposes, especially those applied near the time of planting of the potatoes, should persist long enough in the soil to have an effect on the larvae of the nematode when the larvae pass through the soil from the cysts to the potato roots; and as far as is known, this is the most susceptible phase in the life cycle of the parasite. In neither point of attack, however, has much permanent success been obtained.

Cicero has said that "physicians, when they have found out the cause of a disease, consider that they have also found out the cure". There is a sense, of course, in which this statement is quite true, but there is another in which it is not correct. The primary cause of eelworm disease is known but the cure appears as yet to be far off. The reason may be that the cause is not well enough known nor its implications properly understood and that while investigators were seeking

for methods of control, the zoological and biological aspects of the problem were not fully examined. It may be well therefore, while continuing to employ a rotation of crops where possible and taking precautions to avoid spreading infestation to clean land, to make a tactical withdrawal from the field of chemical control, in order to reconsider the position and discover in the biology of the parasite itself a fresh point of attack which will ensure success for future control measures.

Summary.

1. An outline of the history and symptoms of the disease of potatoes caused by the nematode worm Heterodera schachtii, Schmidt, is given, together with a short account of the life history of the parasite and a brief record of various attempts to control it.

2. Solutions of organic silver compounds such as the proteinate, acetate, benzoate and lactate have a very strong toxic effect on the larvae and contents of the cysts of the worm, this action being dependent on the silver content of the solution.

3. The action of the silver solutions used on the larvae is, with one exception, independent of the organic or inorganic combination of the element.

4. The action of silver nucleinate on larvae and cysts cannot be correlated with that of the other silver compounds and its effect upon larvae appears to reach a maximum at certain intermediate concentrations.

5. The toxic activity of solutions of the other silver compounds on the larvae is maintained even where the silver content is as low as 0.06 mgm. per litre.

6. A hyper-normal motility of the larvae is caused by certain dilute solutions of silver compounds.

7. The toxic action of both silver and mercury

solutions is limited by the presence of potato root excretion which increases the minimum concentration of silver toxic to larvae to about 0.4 mgm. per litre.

8. The time necessary for silver solutions to kill larvae of H. schachtii is longer than that reported for H. marioni.

9. The effect of silver solutions on H. schachtii larvae is stronger than that of certain other compounds used in attempts to control the parasite and records of the action of mercuric chloride on the larvae are noted.

10. The strong toxic action of silver proteinate on the larvae in vitro is not reproduced in pot experiments.

11. The most dilute silver solution to kill the contents of one-year-old cysts of H. schachtii within 3 weeks has a silver content of 40 mgm. per litre.

12. Older cysts are more susceptible to the action of silver solutions, and the contents of 2-3 year old cysts can be killed in 3 weeks by a solution containing 15 mgm. silver per litre.

13. The effect of silver solutions on cyst contents is limited by the presence of potato root excretion, but not to the same extent as their effect on the larvae.

14. In practice the most important factor influencing the action of silver solutions on the contents of cysts is

the soil.

15. Solutions with a low silver content acting for a short time on eelworm cysts cause increased numbers of larvae to emerge from the cysts.

16. Increasing periods of storage of cysts in water tend to produce increases in larval emergence.

17. When silver proteinate is applied to eelworm-infested soil in pots the minimum treatment to have any effect on larval invasion of potato rootlets, as observed by direct methods, is 2.5 cwt. per acre.

18. The optimal application of silver proteinate lies between 10 and 15 cwt. per acre, but although reduction in larval penetration of rootlets and increase in crop are produced, complete control is not effected. Even 40 cwt. per acre does not provide complete control.

19. The potato plant cannot tolerate an application to the soil of more than 10-15 cwt. silver proteinate per acre.

20. The action of these silver compounds on H. schachtii in vitro is satisfactory and encouraging, but their effect in presence of soil is limited and incomplete, rendering their application as a control measure on a field scale uneconomical and therefore impracticable.

NOTES ON THE BIOLOGY OF THE POTATO ROOT EELWORM

1. Larval Emergence from H. schachtii Cysts, extracted at different times from infested soil, and Observations on the Larvae.

One of the most satisfactory measures which can be adopted as a precaution against a severe outbreak of the disease of potatoes caused by the root eelworm Heterodera schachtii, Schmidt, is a simple rotation of crops. This, of course, by no means provides complete control but merely limits the severity of the eelworm attack, since the parasite can survive in a dormant condition in soil for a considerable time and potential infection may remain in a field for at least ten years. Franklin (24) comes to the conclusion that on the whole one-year-old cysts cause the most damage, since the initial invasion of potato roots resulting from larval emergence from these cysts takes place more quickly than from older cysts. Not only so, but the one-year-old cysts have a higher rate of "hatching" which is maintained over a longer period.

Franklin used cysts extracted at the same time from soils in both Yorkshire and Lincolnshire which had not grown potatoes for from one up to eight years, and found that the number of full eggs in the cysts and the numbers of larvae

which they liberated tended to decrease with increasing age of the cysts, but this was not obvious in every case.

Material and Methods.

In the experiments to be described, all the cysts used had been extracted from the same field at Jameston Farm, Maidens, Ayrshire, at different times and stored for different periods under uniform laboratory conditions. Crops of early potatoes had been cultivated annually in the field from which the cysts were taken, for many years up to and including 1940.

In the first experiment conducted during the period January - March 1941, the cyst samples used were extracted in May 1938 and March 1939, i.e. before the digging of the potato crops and deposition of cysts in those years, and in October 1940, i.e. after the season's crop had been dug. Thus the newest cysts in the samples, which contained also cysts produced in previous years, were formed respectively in 1937, 1938, and 1940. It is probable that some of the larvae from the 1938 and possibly some from the 1939 samples had already emerged to penetrate the roots of the potatoes planted before these cysts were extracted.

Six samples of 50 cysts each were placed in potato root excretion and the larval emergence counted over a period of 61 days, after which time practically no larvae were being

liberated.

The experiment was repeated in May - July 1941, and extended to include cysts extracted from the same field in September 1939, February 1940, and May 1941. It would be expected that the "hatches" from cysts of the first two of these three samples mentioned would be of similar proportions and since no potato crop was grown in the field in 1941 it would also be supposed that the larval emergences from the October 1940 and May 1941 samples would be of the same order.

After the cysts had been in contact with root excretion for about 9 days, the larvae which had emerged in each series were extracted and kept in separate beakers. Suspensions of equal numbers of larvae were then transferred to watch glasses at different times and regular observations made, counts being taken of the numbers showing signs of movement. Towards the end of the experiment estimations were made of the numbers of larvae which remained alive.

Experimental Results.

The results of the first experiment are noted on Table I., and indicate a definite and steady decrease in the output of the various cyst samples corresponding to increase in age of the cysts. The difference in reaction of May 1938 cysts and October 1940 cysts to silver has already been mentioned

Table I.

Larval emergence from cysts of different ages. I.

Date of extraction of sample	Larvae liberated from 300 cysts.	Larvae liberated (% of Oct., 1940)	Average number of larvae p. cyst
May, 1938,	1408	14.5	4.69
Mar., 1939,	2400	24.7	8.0
Oct., 1940,	9724	100	32.4

The second experiment provided similar results (Table II.) and except for the "hatch" from February 1940 cysts a gradual decrease in larval emergence corresponding to increasing age of the cysts was again shown. The graphs (Tables III.and IV.) show the respective total "hatches" and the corresponding rates of larval emergence calculated on 5-day periods.

Table II.

Larval emergence from cysts of different ages. II.

Date of extraction of sample	Larvae liberated from 300 cysts.	Larvae liberated (% of Oct., 1940)	Average number of larvae p. cyst
May, 1938,	433	6.8	1.44
Mar., 1939,	830	13.1	2.77
Sept., 1939,	3307	52.1	11.02
Feb., 1940,	787	12.4	2.62
Oct., 1940,	6341	100	21.14
May, 1941,	6194	97.7	20.65

Table III

Graphs of total "hatches" from cysts of different ages.

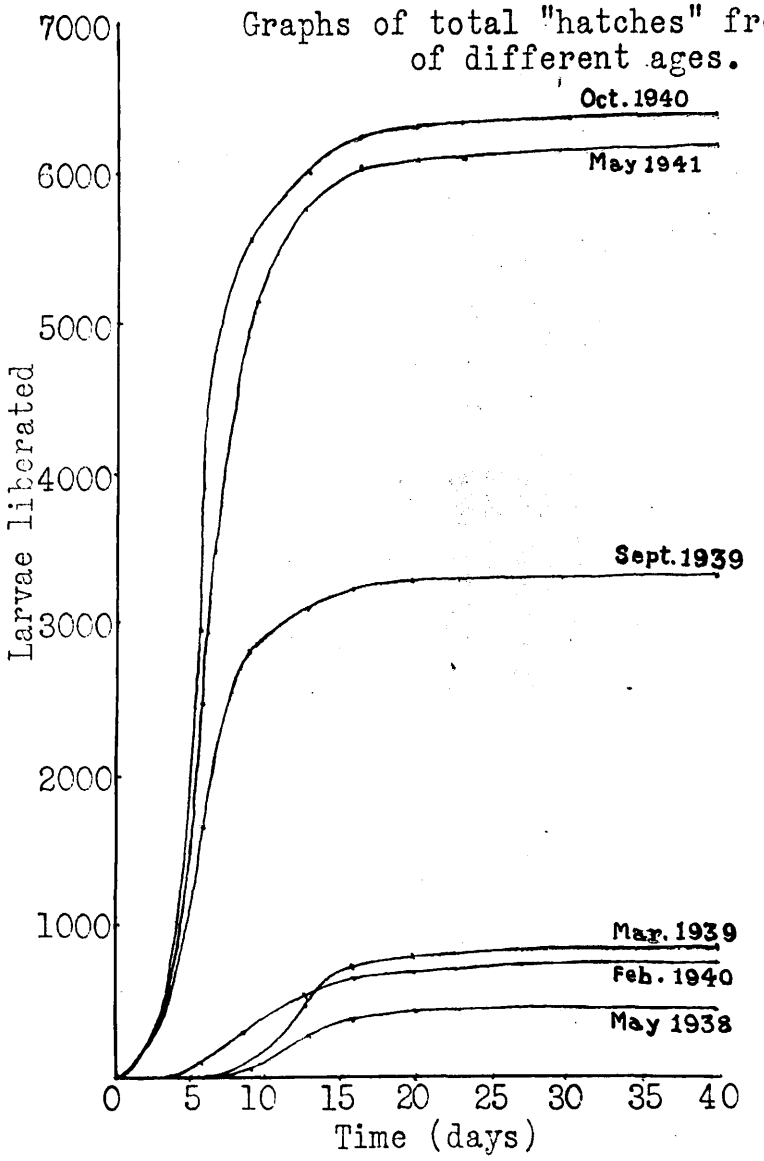
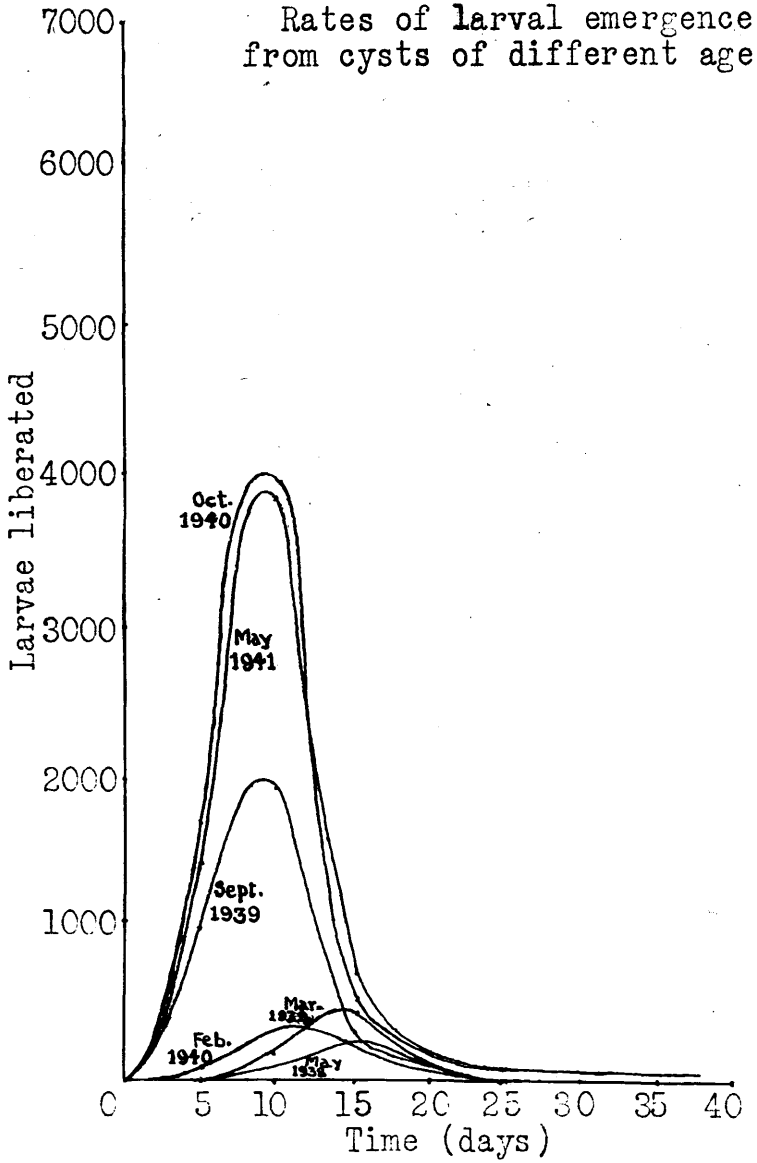


Table IV

Rates of larval emergence from cysts of different ages.



In some of the larval observations there were indications that movement of the larvae from the newer cyst samples was sustained longer than that of larvae from the older cysts, but in the majority of cases no definite order of loss of motility could be established. After about 35 days, most of the larvae became practically colourless, due to their reserve food material having been used up, but this did not appreciably affect the larval motility which had altered but little even 3-4 weeks later. It is interesting to record that in one case, movement was observed after a long period of quiescence, $6\frac{1}{2}$ months after the larvae had emerged from the cyst.

No correlation could be discovered between the longevity of the larvae and the age of the cysts from which they had emerged.

Discussion.

The experiments were designed to determine the mortality rate of the contents of cysts over certain periods of time and it was justifiably assumed that the cysts in the various samples would originally have been capable of producing the same number of larvae.

Larval emergences were smaller in the second experiment than in the first, probably due to the fact that a

different sample of potato root excretion was used, although both samples were believed to be about the same strength. It is unlikely that the difference in the numbers of larvae hatched was caused by the death of part of the cyst contents, since only four months separated the commencement of the two experiments, and in any case, according to Gemmell (32), the "hatches" in the later one which was performed in spring and summer ought to have been greater than those in the earlier experiment which was carried out in late winter.

From the results given, it can be noted that older cysts generally liberate fewer larvae than those more recently formed. Only one sample - February 1940 - was exceptional in this respect. Since the October 1940 and May 1941 samples were the same in all respects except that the latter had remained in the soil seven months longer than the former, and since the larval emergences from both samples were virtually the same, it would naturally be supposed that the "hatches" from the September 1939 and February 1940 samples would also be of a similar magnitude. The only explanation which can be found for the low larval output from the latter cysts is that they were extracted during heavy frost and that this has affected the eggs within the cysts, for, in spite of Baunacke's assertion (1) that exposure to extremely cold conditions had no great effect on cyst contents, experiments described in a later

paper show that in certain cases frost does have a considerable effect.

The increases in larval emergence from newer cysts, seen in the results of both experiments correspond to the intervention of a new potato crop with the resultant formation of a fresh crop of cysts. The cyst contents appear to lose their hatching potentiality comparatively rapidly during the period of storage, e.g. in only one year between September 1939 and October 1940, the decrease in potential infectivity is almost 50%.

From the graphs it can be seen that increasing age of the cysts as well as decreasing larval emergence also causes retardation in the commencement of "hatching" and delay in the time of the maximum rate of emergence. With recently formed cysts the maximum rate of hatching was attained after 9 days in root excretion, but with cysts 2 and 3 years older liberation of larvae began only after 7 days, the maximum rate of hatching being delayed until the 15th.day. These delays are important since as Franklin (24) points out their occurrence in the field may enable the young potato plant to make more growth, so as to overcome the worst effects of eelworm attack.

The differences in the numbers of larvae liberated by samples of cysts of different minimum age were greater than those obtained by Franklin (24), but this may have been because

the cysts used in the work described had been stored for different periods in the laboratory under dry conditions which may have been less suitable for the cyst contents than natural field conditions.

It can be concluded that the findings confirm those of Franklin, but although the decrease in hatching potentiality of older cysts may be explained in terms of relatively rapid mortality of the contents, the slight delays in both larval emergence and the time of the maximum hatching rate of these cysts are not yet fully understood. Further, no differences in mortality rate of the larvae liberated by the cysts of different ages were observed, indicating that those eggs which do hatch after surviving the long periods of storage produce larvae which are unimpaired as regards life and activity.

2. Variations in H. schachtii Cysts from Potatoes grown in different Media.

The potato root eelworm Heterodera schachtii, Schmidt, can thrive under a wide variety of conditions, but obviously some environments are better suited than others for the maximum development of the nematode. The condition of the soil in which the host plant is growing plays an important part in the attack of the parasite on the rootlets which it attempts to invade, and both Triffitt (111) and Reinmuth (79) have recorded that fewer and smaller cysts are formed in heavy than in light soils.

Materials and Methods.

In order to verify these conclusions and to investigate the effect of a medium of pure clay on the development of the parasite as compared with its growth in pure sand, the following experiment was devised. Duplicate pots of eelworm-free sand and clay each of which had received 4 gm. complete fertiliser were inoculated with 10,000 eelworm larvae, and one Epicure seed tuber was planted in each pot. The control pots received no larvae but were otherwise treated in exactly the same way.

When the growth of the resulting potato plants had

ceased and the haulms had died down, the sand and clay from the various pots were thoroughly dried and the newly formed cysts extracted by the Morgan flotation method. Before extraction of the cysts the large masses of clay were ground down in order to liberate the cysts which they contained. The cyst extracts were then dried and cleaned and the total numbers counted, six samples of 50 cysts each taken from each medium, transferred to potato root excretion and the relative larval emergences counted over a period of 93 days. Measurements of the cysts were made using an eye-piece micrometer and the mean diameters calculated.

Pots containing peat moss litter were originally also included in this experiment, but unfortunately there was complete absence of infection in these pots probably because the larvae had been killed by the humic acids present in the peat.

Experimental Results.

The results are shown on Table I. No cysts were found in any of the control pots and the numbers obtained from the sand were 952 and 1016 and from the clay 229 and 114. The smallest of the cysts measured from the clay had a diameter of 0.351 mm. and the largest 0.614 mm. The corresponding figures for cysts from sand were 0.377 mm. and 0.658 mm. The range in larval emergence from samples of 50 cysts produced in sand was

Table I.

Differences in cysts produced in sand and clay.

Medium	Average number of cysts produced per plant	Larval emergence from 300 cysts	Average number of larvae per cyst	Average mean diameter of 120 cysts (mm.)
Sand,	984	27861	92.87	.533 ± .0040
Clay,	174	18921	63.07	.478 ± .0055

from 3353 to 5300 and in those from clay 2195 to 3908.

Liberation of larvae extended over a very long period and did not resemble the usual type of "hatch" in which the rate of emergence, slow at first, rises fairly quickly to a maximum and then decreases more gradually until after 30-40 days it is for all practical purposes zero. In these observations there were three maxima, at the 8th., 29th. and 57th.day respectively. This extremely long and unusual type of hatching is thought to be connected with the relatively recent period between the formation of the cysts and their exposure to potato root excretion.

In both control and inoculated pots the plants grown in sand made stronger growth than the corresponding plants grown in clay. This was indicated by the yields of tubers, which from the control and inoculated plants in sand weighed $5\frac{1}{2}$ oz. and $4\frac{1}{2}$ oz. compared with $3\frac{1}{2}$ oz. and $2\frac{3}{4}$ oz. from the

respective plants in clay.

Discussion.

In this work larval suspensions were used under strictly controlled conditions on two extreme types of soil, instead of the inoculum of cysts used by Triffitt (111) or the method used by Reinmuth (79) of "lightening" the same original heavy soil by the addition of different amounts of sand. It was considered that equivalent larval suspensions would provide a more uniform initial source of infection.

Four facts are outstanding from the experiment. From an equivalent larval inoculum fewer cysts are formed on plants grown in clay than in sand, the cysts formed in the clay being smaller in size and producing fewer larvae. The other fact is that the potato plants grown in clay are themselves smaller than those grown in sand.

It is hardly likely that the smaller number of cysts formed in the clay was due to such a great invasion of some of the first-formed rootlets that these were killed causing the death of the larvae in them. If this had been the case, it would have occurred also in the sand, and this theory cannot account for the extreme difference in the numbers of cysts formed in the two media.

The smaller number of cysts formed in the clay must

thus be taken as an indication of the difficulty experienced by the larvae and adult male worms in penetrating this medium to reach the potato roots, although Triffitt (111) suggests that lack of aeration may play an important part. Considering the density of the clay, the number of cysts that were formed is in itself a demonstration of the penetrative powers of the larvae.

The general decrease in size of cysts produced in clay compared with those formed in sand is most probably due to the increased pressure of the denser medium round the roots of the plant, a purely physical reason. On the other hand there is a possibility of a physiological explanation. The plants themselves which were grown in clay were in all respects somewhat smaller than those in sand. Consequently the smaller plants might not have been able to provide the parasite with sufficient food material with the result that smaller cysts were formed. This view cannot, however, be consistently maintained since relatively large cysts have been observed on small potato plants grown in sand.

Another possible explanation of the reduction in size of the cysts in clay may be that the main larval invasion of the rootlets may have taken place at a later stage in the life of the host plants than occurs in sand, with the result that most of the cysts formed in clay may not have had a chance

to attain larger dimensions. This is not thought to be likely although there are no facts to invalidate this explanation.

The experiment also showed that larger numbers of larvae emerged from larger cysts formed in sand than from the smaller ones produced in clay. This agrees with the relationship between cyst size and egg content shown by Triffitt (109) and also with the relationship between larval emergence and cyst size observed by Gemmell (32). Whether cyst size in this experiment is determined by the number of eggs in the cyst or vice versa appears to depend on whether we regard the phenomenon as due to physiological or physical agencies. In other words, if the density of the clay primarily affects the plant whose limited growth tends to reduce the production of eelworm eggs, then this will in turn cause a reduction in cyst size. If, however, the pressure of the clay round the roots of the plant is experienced to any extent by the young cysts, their resultant limitation in size will determine their egg content. Although both lines of action may be involved in certain cases, from the evidence of Reinmuth (79) and Triffitt (111) it is believed that the density of the medium is the primary cause of the reduction in cyst size which results in the formation of fewer eggs and liberation of fewer larvae.

3. The Reactions of Larvae and Cysts of H. schachtii to Low Temperatures.

In 1901, Karpinski (51) attempted to control the beet strain of H. schachtii by methods of deep ploughing during winter, in order that the frost might attack and damage the cysts and any free larvae present in the soil. Since infestation in the field is present at levels much deeper than that to which the plough penetrates (1), and since the resistance of the parasite to frost appeared to be considerable, Karpinski's method did not meet with much success. Strubell (100) and other workers had suggested the susceptibility of H. schachtii to certain degrees of frost, but Baunacke (1) showed that the free larvae of the beet eelworm could withstand even repeated freezing at -8°C. and that the embryonic larvae in the egg cases were still alive after being kept in an ice block at -9°C. for 6 days. Baunacke further claimed that after exposing cysts to a temperature of -9°C. for 6 days their ability to liberate larvae was undiminished. If the larvae of the potato strain of H. schachtii can withstand such low temperatures, it is not difficult to understand how they can overwinter in the soil, a fact which was shown by Franklin (23).

Experiments with Cysts.

These experiments were performed in two series at different times, but in both cases cysts from the same sample were used. The first series was carried out in winter and three conditions of cysts were employed (a) dry, (b) in water immediately before the commencement of the experiment, and (c) in water for 6 days. Samples of cysts in these conditions were placed in watch-glasses inside petri-dishes and kept outside overnight. The minimum temperature to which they were exposed was -7.2°C. Similar samples were placed for the same period in an incubator at 20°C. Four lots of 25 cysts each were then transferred from all the frozen and control samples into potato root excretion and the larval emergence observed over a period of 54 days.

The second series of experiments with cysts was undertaken in summer and the cysts, again in the same conditions as previously, were frozen in a refrigerator. Two different times of exposure to low temperature were employed, some cysts being kept at -10°C. for 24 hours and others at -8°C. for 7 days. The "hatching" of the cyst samples was carried out as before.

Experimental Results.

A definite decrease in larval emergence can be noted

Table I.

Larval emergence from control cysts and cysts frozen under natural conditions.

Previous condition of Cysts.	Emergence from 100 cysts.		Emergence from frozen cysts (% of control)
	20°C.(control)	-7.2°C. overnight.	
Dry,	7396	2242	30.3
In water,	3248	2197	67.7
In water 6 days,	3926	1781	45.4

from those cysts which had been frozen (Table I.), and this appears to take place whether the cysts were dry or had been previously soaked, although some of the individual numerical "hatches" were not very consistent. That this decrease in larval emergence was not caused by merely a delay in hatching was shown by the fact that six months later the number of larvae escaping from similar samples of the cysts which had been kept dry and exposed to low temperatures was still only 47.8% of the control which was kept at 20°C.

The results shown on Table II. and III. are entirely different from those on Table I. In spite of the fact that the temperatures were lower and the times of exposure longer than in the first experiment, in only one instance can an appreciable decrease be observed. It is strange also that increases in the number of larvae liberated should be recorded

Table II.

Larval emergence from control cysts and cysts frozen in refrigerator I.

Previous Condition of Cysts.	Emergence from 100 cysts.		Emergence from frozen cysts (% of control)
	20°C.(control)	-10°C.for 24 hours.	
Dry,	2604	6905	265
In water,	2297	3847	167.5
In water 7 days,	2910	2990	102.7

Table III.

Larval emergence from control cysts and cysts frozen in refrigerator II.

Previous Condition of Cysts.	Emergence from 100 cysts.		Emergence from frozen cysts (% of control)
	20°C.(control)	-8°C. for 7 days.	
Dry,	7373	6405	86.9
In water,	2565	879	34.5
In water 7 days,	3415	3160	92.5

from cysts kept at -10°C. for 24 hours and that control cysts which had been stored dry should produce in both experiments more larvae than those kept in water.

Discussion

It is difficult to explain the differences in the larval emergence between the two experiments. It may have been because an insufficient number of replications was employed to counteract the individual variations in the cysts. It is not improbable also that if the emergence rate from cysts tends to fall off in winter as Triffitt (111) and Gemmell (32) have reported, the vitality of the cyst contents at this period of the year is lowered. In this case the contents may be more susceptible to frost during winter than to the same low temperatures in summer. It will be remembered that in the experiments with cyst samples of different minimum ages, the cause of the low larval emergence from cysts taken from the soil in February 1940 was believed to be the very hard frost at the time of extraction.

No significant differences were observed in the effect of frost on cysts in different conditions. It might be supposed that cysts having been newly placed in water and floating on the surface would be crushed when the water changed to ice. It is also reasonable to suppose that when cysts which have been thoroughly soaked with water are exposed to frost and the water which they have absorbed freezes, the cyst wall will rupture or else injury will be suffered by the egg mass. In these experiments, however, no sign of any cysts damaged from

these causes were observed. Repeated freezing and thawing was not attempted with the cysts and it is possible that the action of low temperatures in this manner may be more lethal to the contents.

From the results which have been noted, it can be stated that exposure of cysts to freezing conditions may in some cases destroy part of the contents of the cysts, but even temperatures lower than those which occur in the soil (except perhaps in the surface layer), if they do affect the cysts at all, do not provide complete control of the parasite.

Experiments with Larvae.

Freezing experiments with free larvae were also performed in two series. In the first, use was made of naturally cold winter conditions and in the other, carried out in summer, a refrigerator provided the low temperatures. Potato root excretion suspensions were used, the watch-glasses in each comparable set containing equivalent numbers of larvae. The controls were kept in an incubator at 20°C. while the test larvae were subjected to various temperatures, and in cases where no effect was produced the freezing was repeated. The total number of larvae in each watch-glass was counted and also the numbers which showed movement both before and after treatment. Finally after allowing a suitable time for the recovery

of the frozen larvae, all the sets of treated and control larvae were washed on pots of sand in which a sprouted potato tuber had been planted, to determine whether the larvae which had been frozen were dead and merely quiescent. (These experiments were performed before definite criteria of death had been found or established.) When the resulting potato plants had died down the cysts produced in each pot were extracted and counted.

Experimental Results.

Table IV. shows in a concise form the freezing treatments and their corresponding effects upon the free larvae. The temperatures recorded in every case except those of the controls are the minimum to which the larvae were subjected in being kept outside overnight.

The results of the experiments where larvae were frozen in a refrigerator bear out in a significant manner those recorded in Table IV., and are seen in a condensed form in Table V. The last three results in this table refer to repeated exposure of these larvae to low temperatures. After 1 hour at -6°C. no freezing occurred and no check in the motility of the larvae was observed. Not much difference was produced after these larvae were kept for 2 hours at -8°C. but final subjection to -10°C. for 3 hours brought about the death

Table IV.

Effect of overnight freezing upon potato eelworm larvae.

Total number of larvae	Number moving before treatment	Treatment (°C)	Number moving after treatment	Subsequent treatment (°C)	Final number moving	Number of cysts produced.
96	-		0		0	
113	-	-5.6	0	-	0	0
110	-	(all[x])	1		0	
118	-		0		0	
149	-		100		44	
89	-	20	60		33	83
101	-		72	-	45	
124	-		62		32	
81	-		32		0	
271	187	-2.2	125	-16	0	0
100	59	(none[x])	61	(all[x])	1	
184	116		109		0	
161	102		56		15	
169	103	20	64	20	26	74
225	134		101		50	
184	124		97		53	
263	151		217		0	
270	128	-5.6	170	-16	0	4
256	138[x]	(one[x])	16	(all[x])	0	
290	127		142		0	
291	113		-		186	
276	106	20	-	20	194	52
263	92		-		144	
301	110		-		107	

[x] Number of watch-glasses frozen.

Table V.

Effect upon potato eelworm larvae of freezing in a refrigerator.

Treatment (°C.)	Duration of treatment (hours)	Number of larvae	Final number of larvae moving.		Number of cysts produced.	
			Treated	Control	Treated	Control
-10	24	3400	0	2400	0	325
-8	48	1500	0	1100	10	159
-8	6	800	2	400	6	61
-8	4	1600	5	1100	2	-
-6	1	)				
-8	2	)[x] 800	17	540	1	-
-10	3	)				

[x] See text.

of most of the larvae.

It was noticed in both experiments that the free larvae could withstand low temperatures where complete freezing of the medium did not take place. Several instances were observed where the larval suspension had frozen completely except for a small part at the base of the watch-glass and when the medium had been thawed and time given for the larvae to recover, not a few of those in the unfrozen area were moving, while those around the edge of the liquid were motionless and

had every appearance of being dead.

Discussion.

The results of these experiments indicate that the free larvae of the potato strain of H. schachtii are more susceptible to the effects of freezing than would be supposed from Baunacke's experiments (1) with the beet strain, where exposure to a temperature of -8°C. in ice blocks for 24 hours had no detrimental effect on the larvae. In contrast to this, most of the larvae of the potato strain were killed after 4 hours' subjection to this temperature but whether this effect is due to differences in the strain of H. schachtii or to dissimilar conditions cannot be stated.

Even by placing larvae of the potato race outside overnight, exposed to ordinary winter frost where the minimum temperature was -5.6°C., complete control was for all practical purposes achieved, provided that the larval suspension was frozen through. The free larvae were killed when they were completely surrounded by ice, while they were capable of withstanding the same and slightly lower temperatures if for some reason only partial freezing of the medium was effected. It is possible then that the cause of the death of the larvae at low temperatures is the pressure of the expansion which occurs when water changes to ice, but whatever the reason may be, the

results obtained in these experiments and those found by Baunacke (1) do not coincide.

Conclusions

Although the results of the action of frost on cysts of H. schachtii tended to be conflicting, indications were obtained that a lethal effect is produced under certain conditions. The influence of frost on the larvae of the nematode is very marked and may occur at temperatures which are quite common during an average British winter. In view of the fact that free larvae can overwinter in the soil (23), it must be supposed that they do this at some depth since larvae in the surface layer would probably be killed by severe frost. If there was therefore any risk of damage to potato plants by these free larvae, it would be advisable to undertake deep ploughing to allow the frost to kill the larvae, while at the same time it might also act on the contents of the cysts.

4. Determination of Death in H. schachtii Larvae.[x]

One of the major difficulties in the work of helminthologists has been to differentiate between living and dead nematodes. According to Lapage (57), most of those who have studied the metabolism of these worms have taken their failure to move, either as a result of or without the action of a stimulus, as an indication that they are not alive.

Normally under suitable conditions, most of the larvae of H. schachtii are in active motion, but it frequently happens that they lie quite still for a relatively long period and then, for some undiscovered reason, recommence active movement. They may also show no sign of movement when observed in watch-glasses, but yet be able to penetrate potato rootlets. Thus it is exceedingly difficult to distinguish whether larvae are in a state of dormancy or whether they are dead. According to Baunacke (1), who made a study of the beet strain of this eelworm, that part of the larval body near the oral end, which is almost completely hyaline in the living larva becomes granular after death occurs. After treatment with certain solutions or after exposure to certain degrees of heat, larvae of the potato strain of H. schachtii have been observed to be

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[x] The substance of this paper was published as a note in "Nature", 1941, No.148, p.728.

in a condition similar to that ascribed by Baunacke to a dead larvae. The probability is that coagulation or precipitation of the protoplasmic colloids has taken place and this imparts a uniform opacity to the dead larva. Other larvae have been seen to show a peculiar distention of the body wall near the higher end of the intestine, while the body contents in this region had become displaced. This condition, resembling the effect of plasmolysis, does not appear to be reversible and is followed later by the granularity described above.

Although it is known that living nematodes may in certain cases have the appearance of being dead, it was ascertained that larvae of the potato strain of H. schachtii in the above described conditions could not be induced to form cysts on the roots of potato plants. Such larvae were considered to be dead.

It was also determined that dead larvae of both these types could be more clearly distinguished from living larvae, by staining with a solution of 0.025 gm. iodine in 100 c.c. 1% potassium iodide solution. The most satisfactory procedure was to use 5 drops of this solution in 2 c.c. of a larval suspension. The suspensions employed contained some larvae in active motion and some considered to be dead, and the latter absorbed the iodine and were stained within a few minutes, being best observed after between 10 and 20 minutes. Those

larvae which were moving and thus known to be alive originally became motionless after a few minutes, but yet retained the appearance of living larvae for several hours and did not absorb the stain. They were killed, however, when left in this solution overnight.

The iodine penetrates the dead larvae through the mouth and by careful observation a yellowish coloration may be seen starting at this end and gradually permeating the whole body.

Larvae which exhibit a slight granularity in the upper region of the body, i.e. larvae which according to Baunacke's criterion are newly dead, do not take up the iodine immediately, but they do stain after being allowed to stand for 24-48 hours before applying the solution.

First-stage larvae which had been kept in potato root excretion solutions for several weeks, and which by using up all their reserve food material had become practically colourless and clear except for a small granular portion near the mouth, absorbed iodine only slightly in this region while the rest of the body remained almost unstained. This is apparently due to the absence of intestinal contents in the larvae which had been in a free state for several weeks.

Hence larvae of the potato strain of H. schachtii which appear granular in that part of the body which in the

living larvae is hyaline also show an internal absorption of iodine and may be considered as dead. The two main disadvantages of the staining process are that iodine itself eventually has a toxic effect and that the staining does not in some cases take place immediately after death.

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Investigations of Anatomical and Biochemical Aspects of Leguminous Root Nodules.

The research to be described was begun in 1938 in the Botany Dept., Glasgow University, under the supervision of Dr.G.Bond, but unfortunately it was found impossible to complete some of the work undertaken, owing to the receipt of an appointment in the West of Scotland Agricultural College, Auchincruive.

Work was commenced on two aspects of the root nodules of Leguminous plants, namely (A) Observations on the Structure of Leguminous Root Nodules, and (B) The Excretion of Nitrogenous Substances from Root Nodules. The former was continued by Miss Helen L.Frazer whose paper on this subject is in process of publication and therefore only a brief outline is presented below. Part of the latter work was completed by Dr.Bond and the results included in a paper "Symbiosis of Leguminous Plants and Nodule Bacteria. II. Further Observations ..... " (Ann.Bot.N.S.V., 20, Oct., 1941). Further experiments on excretion of nitrogen were later carried out and completed at Auchincruive, observations on this work also being included in Dr.Bond's paper.

A. Observations on the Structure of Leguminous Root Nodules.

The desirability of this investigation was suggested by consideration of the results of experiments carried out in recent years by Virtanen (16) on the excretion of fixed nitrogen from nodules into the rooting medium. In some of these, Virtanen reported that excretion was greatest from nodules on young plants, but although he secured abundant excretion from pea nodules, other workers failed to obtain similar results with other legumes (e.g. Bond (3) with soya bean). It appeared possible that these results might find their explanation in anatomical differences particularly in the distribution of suberised tissues such as endodermis and cork within the nodule. Besides its relation to the excretion phenomenon, consideration of the extent to which the structure of the nodule facilitates exchange of material with the environment seemed likely to be of interest in connection with the general activities of the nodule.

Examinations of the anatomy of nodules have been made by Spratt (12), Brenchley and Thornton (6), Dangeard (7), Thornton and Rudorf (14), Bieberdorf (1) and others, but in the literature no special attention has been paid to the distribution of suberised tissues and to its effect on the function of the nodule.

Methods.

Microtome sections were prepared from nodules of the following legumes: Pisum sativum, L. (garden pea), Vicia faba, L. (broad bean), Glycine soja, Sieb. et Zucc. (soya bean), Phaseolus multiflorus, Willd. (runner bean), Trifolium sp. (clover) and Lupinus sp. (garden hybrid lupin). Nodules of different sizes, taken from plants at different stages of development, were preserved in 70% alcohol and sections cut serially in various directions. The wax ribbons were stretched on a glass slide and attached to another slide by a film of a collodion-clove oil mixture. (Bond (2)). After being cleared in Eau-de-Javelle, the sections were either mounted directly in Gentian violet-glycerine jelly, or stained in warm Sudan III glycerine and mounted in glycerine jelly. Sudan III. was used for the detection of cutinised and suberised tissues and Gentian violet for lignified structures such as the Caspary strip, although it also tended to stain purely suberised tissues. Ammoniacal basic fuchsin was also used as a lignin stain in a few cases, the preparations being then mounted in Canada balsam.

Conclusions.

This work had to be discontinued before a complete detailed examination of the preparations could be made, but

from the account of the investigation by Miss Frazer, it is clear that the distribution of the endodermis is such as to impose a definite restriction on the diffusion of solutes and gases into, or out of, the central tissues of the nodule. This was confirmed by means of experiments on diffusion of dyes into the nodule.

B. The Excretion of Nitrogenous Substances from Root Nodules.

It has long been a common practice to grow mixed crops of leguminous and non-leguminous plants and several writers have noted that this system was adopted even in the very primitive agricultural economies of many tribes and peoples from different parts of the world. It was only in 1892, however, when La Flize (9) suggested that the associated crop made use of nitrogen fixed by the legume, that the first hint was given as to the true nature of the benefit derived by the non-legume. Lipman (10), in 1912, advanced the theory that this was due to the excretion of nitrogenous compounds from the leguminous plant, but little attention was paid to the results of his experiments.

As a result of work commenced in Finland in 1927 and continued in the following years, Virtanen (16) again postulated the excretion hypothesis and his further researches

provided conclusive evidence that, under certain conditions, several legumes can excrete nitrogenous compounds from the root nodules and that these excreted substances can be taken up by the non-legume which is grown in association. Virtanen and his collaborators also showed that the nitrogen of the excreted compounds, which they found to be primarily aspartic acid and beta-alanine, amounted in some cases to as much as 80% of the total nitrogen fixed by the plant.

Such strong evidence of excretion has not been universally confirmed by other workers even when repeating Virtanen's experiments as exactly as possible, and although some, e.g. Thornton and Nicol (13), reported positive evidence in certain cases, the investigations of Bond (4), Ludwig and Allison (11) and Wilson (20) have failed to show excretion to any significant degree.

According to Virtanen (15), various factors such as the bacterial strain, the composition of the growth medium, its absorptive capacity and nitrate content and the number of nodules formed on the host plant all control the extent of excretion. Density of planting of the legumes (18) and aeration of the growth medium (19) are also claimed as having an effect on excretion. Most of these factors have been studied by other workers, but while it has been shown that some do play a part, no set of conditions has yet been

discovered under which the excretion phenomenon may regularly be observed, except in Finland where a successful combination of environmental factors and experimental arrangements appears to obtain.

Wilson and Wyss (21) working in Madison, Wisconsin, suggested that the relation between photosynthesis and nitrogen fixation as controlled by environment may be a dominant factor and that in cases where excretion occurs, carbohydrate synthesis is sufficiently rapid to allow a high rate of fixation of free nitrogen but not high enough to convert this fixed nitrogen into new tissue. Consequently an excess of nitrogenous compounds accumulates in the nodules and part of this is excreted. From subsequent work at the above station, it was shown that factors such as length of day, relatively low temperature and shade in summer which control the relationship between the rates of photosynthesis and nitrogen fixation, also influence the occurrence of such excretion as was obtained. On the other hand, Bond and Boyes (5) found that in Glasgow, under conditions of comparatively little direct sunlight, no excretion occurred, but they indicated the possibility that the plants in these experiments may have been exposed to an insufficient amount of sunlight.

The evidence of excretion where it has been obtained by other investigators has never been on the same scale as

that found in Virtanen's experiments. This is apparently due to a large extent to variable local climatic conditions and since other factors which appear to affect the amount of excretion can be controlled and rendered uniform in the different investigations, certain workers including Wilson (20) have concluded that the problem seems to be one of locality.

Experimental Methods and Data.

Glasgow experiment. Virtanen (17) has shown that in Helsinki it is possible to obtain excretion of nitrogenous compounds even from plants grown in winter under artificial light. Such conditions may be relatively easily repeated, thus eliminating certain meteorological factors which vary between the different stations.

An experiment under conditions as far as possible similar to those obtaining in Virtanen's investigations was commenced on January 17, 1939, being taken over by Dr.Bond in March and continued until May 29, 1939. The amount of natural light which the plants received was relatively small in the early months and was supplemented for 18 hours per day during the whole experiment by illumination from a 1000 watt lamp which, suspended 30-50 cm. above the plants, provided light of an intensity of 1200 ft. candles. This is comparable to the natural light of a moderately cloudy summer day. In order that

the heat from the lamp should not unduly raise the temperature of the environment in which the plants were growing, a glass bottomed trough through which a continuous current of water flowed was fitted to the reflector of the lamp.

Three pea plants (Pisum sativum, L.) associated with three barley plants (var.Chevalier) were grown in glazed pots containing 1.6 kg. sterilised sand which had received an application of a nitrogen-free modified Hiltner culture solution. The pea and barley seeds had originally been sterilised and the peas then inoculated with a suitable strain of nodule-forming bacterium. Four varieties of pea - Torstag, Concordia (both supplied by Prof.Virtanen), Gladstone and Maple - were used in conjunction with two strains of Rhizobium leguminosarum - '317', a strain from Wisconsin and 'HX', a Finnish strain. The sand employed as a rooting medium was also of two types, fine quartz and superfine red sand as used by Bond and Boyes. The pots and plants occasionally received further applications of culture solution and were kept at standard weights by watering with distilled water.

The sand analyses as well as sterilisation and inoculation techniques and the composition of the culture solution will be described in connection with the Auchincruive experiments which involved practically the same methods.

Photographs of some of the plants are included by

permission of Dr.Bond (figs.I. and II.). From these it may be observed that the growth and development of the pea plants was on a highly satisfactory scale, as was also their nitrogen fixation which in some pots averaged 70 mgm. nitrogen per plant. Only a small trace of excretion was obtained, being shown by the Concordia peas with HX. bacterial strain growing in the superfine red sand, the nitrogen content of the three associated barley plants being 8.8 mg. compared with 5 mg. in the controls.

It has been shown at Wisconsin that a long photo-period and fairly low temperature such as obtained during this work, are conditions under which excretion is found to occur. From the experiment which has been described, however, it can be deduced that these factors, per se, will not cause excretion under Glasgow conditions, and that the presence of other agencies is necessary for the phenomenon to take place.

Auchincruive Experiments. It was suggested by Dr.Bond that it would be of interest to conduct experiments on excretion at the West of Scotland Agricultural College, Auchincruive, Ayrshire, since the general conditions for plant growth, and in particular the amount of direct sunshine, are considerably more favourable there than in the vicinity of Glasgow.

New unglazed pots of 10 inch diameter containing 8 kg. red sand were used. This sand which was obtained locally was

Fig.I. Glasgow Experiment. Concordia peas inoculated with HX strain of bacterium growing in superfine red sand, associated with Chevalier barley. A small trace of excretion was obtained in these pots. The control barley plants are also shown.

Fig.II. Glasgow Experiment. Concordia peas inoculated with HX strain of bacterium growing in association with Chevalier barley in quartz sand. The control barley plants are also shown. The growth in this medium is not so satisfactory as in the superfine red sand.

practically sterile and nitrogen-free, judging from the symptoms of nitrogen deficiency in plants grown in this medium even for a relatively short time. Mechanical analysis of this sand, together with those used in the Glasgow experiment and also of the medium with which Virtanen obtained many positive signs of excretion are shown on Table I.

Table I.

Mechanical analyses of rooting media.

Particle size. (mm.)	Glasgow Expt. fine quartz. (%)	Glasgow Expt. superfine red sand (%)	Auchincruive Expt. (%)	Virtanen. Finnish quartz. (%)
> 2.00	0	0	2	0
2.00 - 1.00	0	0	2	0
1.00 - 0.50	1	0	11	0
0.50 - 0.25	30	0	21	9
0.25 - 0.10	68	62	26	56
0.10 - 0.05	1	28	38	30
< 0.05	0	10	-	5

Modified Hiltner culture solution (8) was applied to the sand in each pot at the rate of 9 oz. per 3 lb.sand. This solution was made up as follows:- Water 2 litres, KCl 0.5 gm., $Ca_3(PO_4)_2$ 0.5 gm., Ca $SO_4.2H_2O$ 0.5 gm., $MgSO_4.7H_2O$ 0.78 gm.,

$CaCO_3$ 2 gm., $FeCl_3$ (5% soln.) 6 drops.

In general the technique was similar to that employed by Bond (3). The pea seeds were initially sterilised by being placed for 2 minutes in absolute alcohol, washed with sterile water and then transferred to a 0.1% solution of mercuric chloride for 6 minutes. After being washed again they were allowed to imbibe water overnight. Bacterial suspensions in sterile water were made up from cultures of two strains - 'HX' the Finnish strain used by Virtanen and '317' from the Wisconsin collection - and the peas inoculated. Twelve were planted per pot but after germination only six were allowed to remain. A few days after the peas had been sown, sterilised barley seeds were sown in spare pots and when they had germinated six were transplanted beside the young pea plants in each pot, twelve barley plants being allowed to grow alone in the control pots.

When it was considered that the peas had become self-supporting, the cotyledons were excised, so that no nitrogen from rotting plant material should be available to the barley. The pots were watered regularly and at intervals received 100 cc. culture solution. They were kept in a cold frame and obtained the maximum amount of sunshine, being covered only during the night and in the event of rain.

When the plants had finished their growth, the number of barley grains produced per pot was counted. The

contents of the pots were then tipped out, the barley and pea plants including roots separated, dried and ground, and their dry weights obtained. Small samples of the plant material were analysed by the salicylic acid - Kjeldahl method, and the total nitrogen content of peas and barley determined for each pot or series of pots.

Experiment I. This experiment, the results of which are presented on Table II., was conducted during the period April 21 - August 25, 1939, using peas of the varieties Concordia, Gladstone and Torstag in association with Chevalier barley. A suitable supply of distilled water was not available and the culture solution was made up with ordinary tap water which was also used to water the plants. The small additional supply of nitrogen which the plants obtained in this way undoubtedly accounts for the unusually high nitrogen content of the control barley plants, and the interpretation of the experiment is thereby complicated.

It was observed that although all the barley plants made good growth, the peas were smaller than those in the Glasgow experiment. There is some indication, however, from Table II., that excretion of fixed nitrogen and uptake of this by the barley did occur, since in each case the average figure for the barley growing with the peas is greater than that of the controls.

Table II.

Analyses of Pots with six peas and six barley plants.

Pot number	Pea Variety	Bacterial strain	Dry wt. of peas (gm.p. pot)	Nitrogen content of peas (mgm. p.pot)	Dry wt. of barley (gm.p. pot)	Barley grains formed per pot	Average Nitrogen content of barley (mgm. p.pot)
1	Concordia	HX	7.9		5.1	41	
2	"	"	9.7	136.5	5.1	36	72.3
3	"	"	11.3		8.7	96	
4	Concordia	317	14.7	266.5	12.9	113	
5	"	"	9.2	146.9	8.7	65	92.9
6	"	"	9.1	145.5	8.8	77	
7	Gladstone	HX	5.4	96.5	8.0	65	
8	"	"	7.7	126.0	8.7	69	71.7
9	Torstag	HX	6.6	102.5	7.9	76	
10	"	"	5.4	84.6	8.5	73	72.7
11	-	-	-	-	7.1[x]	64.5[x]	48.8[x]
12	-	-	-	-	8.5[x]	69[x]	58.8[x]

[x] 12 barley plants in control pots. Thus half figure obtained is shown.

Experiment II. In an attempt to confirm the indication of excretion obtained in the previous experiment, a further series of pots was set up in which glass-distilled, nitrogen-

free water was utilised. In this experiment, which was carried out from May 7 - September 12, 1940, the two bacterial strains previously used were again employed, but only one variety of pea, namely Concordia, was grown since this variety made best growth and gave promise of the most satisfactory results in the first experiment.

The plants were not so large as those in the first experiment and the photographs (figs.III.and IV.) show that the peas were smaller than even corresponding plants grown under apparently less favourable conditions in Glasgow (4). From the detailed results presented on Table III., it can be seen that in only three pots did the barley have a significantly greater nitrogen content than the controls whose average nitrogen content was 16.9 mgm., the increases being respectively 22.6 mgm., 14.4 mgm. and 11.5 mgm.

Discussion.

Only the results of the Auchincruive experiments will be considered here since only this work was personally brought to a conclusion.

It has been mentioned that in the first experiment the plants received tap water, the nitrogen of which was responsible for their relatively high nitrogen content which can be recognised in the control barley and on this account the

Fig.III. Second Auchincruive Experiment. Concordia peas (HX bacterial strain) growing in association with Chevalier barley. Both control pots of barley were similar, but only one (10) is shown. Indications of excretion were obtained from pots 2 and 3.

Fig.IV. Second Auchincruive Experiment. Concordia peas (317 bacterial strain) growing in association with Chevalier barlay. One control pot of barley (10) is shown. An indication of excretion was obtained from pot 6.

Table III.

Analyses of pots with six Concordia peas and six barley plants.

Pot number	Bacterial strain	Dry wt. of peas (gm.per pot)	Average nitrogen content of peas (mgm.per pot)	Dry wt. of barley (gm.p. pot)	Barley grains formed p.pot.	Nitrogen content of barley (mgm. p. pot)
1	HX	5.1		1.6	8	19.4
2	"	7.3	111.2	3.6	24	39.5
3	"	9.0		3.1	23	31.3
4	"	4.8		1.7	8	20.4
5	317	4.2		1.6	7	18.4
6	"	11.4	106.9	2.4	13	28.4
7	"	5.1		1.5	14	19.3
8	"	4.3		1.7	9	20.4
9	-	-		1.3^{x}	7.5^{x}	14.8^{x}
10	-	-		1.8^{x}	10^{x}	18.9^{x}

^{x} 12 barley plants in control pots. Thus half figure obtained is shown.

validity of the results of this experiment may be questioned. Nevertheless the barley plants growing in association with the peas received the same treatment as the controls and their average nitrogen contents are in all cases greater, indicating that a certain amount of excretion did occur.

These results were not completely confirmed in the second experiment where despite favourable growth conditions both pea and barley plants were much smaller than in the first experiment and smaller also than those grown in apparently less suitable conditions in Glasgow. This can be seen not only from the photographs but also from the dry weights and relative numbers of barley grains produced. Evidence of excretion was observed in three of the pots but although the uptake of nitrogen by the barley was greater than that found in Glasgow experiments, it was still not comparable with typical Finnish results.

The sand employed was only slightly coarser than that used by Virtanen (Table I.) and therefore should have been favourable for the detection of excretion. The aeration of the root systems in the porous pots should also have been adequate and the plants received the maximum amount of sunlight. In this last respect especially, the approximation to Finnish conditions should have favoured the occurrence of excretion to a considerable extent.

It may be noteworthy that the positive evidence of excretion which was obtained was found in pots where the nitrogen fixed and growth made by the peas was greater than by those plants which gave no sign of excretion, i.e., larger pea plants were associated with larger barley plants. According to Virtanen and others, including Wilson and Burton (22), this should not be the case, since it has been shown that in legumes

which do excrete nitrogen, growth and fixation is much less than in non-excreting plants. This may raise the question as to whether the nitrogen increases registered were merely due to external and unknown agencies, but there seems to be no other reason to doubt that they were actually the result of excretion on the part of the pea plants.

It is possible that excretion on an extensive scale did take place and that the products remained in the sand without being absorbed by either peas or barley. Since no sand analyses were performed, it cannot be stated whether this did occur or not, but previous analyses of rooting media in investigations where no evidence of excretion was forthcoming produced negative results (3).

It may thus be concluded that there was some positive evidence of excretion especially where tap water was used. This, however, introduced another source of nitrogen and consequently true excretion in this case cannot be claimed with certainty. In only three cases at most, where no additional nitrogen was obtained by the plants, did excretion reach a relatively significant level but even here it was not comparable to the results of Virtanen's investigations under similar conditions. Thus in spite of an apparently favourable climatic environment and experimental arrangements controlled as far as possible, no conclusive manifestations of extensive excretion

of nitrogenous substances from the leguminous plants under investigation were observed.

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The Control of Blind Seed Disease of Ryegrass.

Blind seed disease of ryegrass was first reported by Hyde (4) in 1938 from New Zealand. After an examination of samples of seed which showed a very low percentage germination, the presence of fungal spores was found to be a constant feature of the surface of the caryopsis of those seeds which failed to germinate.

In the field, it was noticed that the florets of certain ryegrass flowers were covered with a pinkish slime, which on further investigation was found to be different from that produced by Ergot (Claviceps purpurea), and contained a mass of conidia similar to those which occurred on the non-germinable ryegrass seeds. Inoculation of healthy ryegrass flowers with a spore suspension of the conidial slime had the primary effect of reproducing the slime on the treated florets and resulted in the formation of typical 'blind' seeds.

Further work by Neill & Hyde (6) has elucidated the main points in the life history of the 'blind seed fungus'.

Few infected seeds are distinguishable from healthy ones except under magnification, but when viewed microscopically by direct light, the diseased seeds appear opaque with dull reddish-purple areas of conidia on the surface of the caryopsis. Such seeds having fallen to the ground usually

fail to germinate and remain in this condition over winter. It is customary to sow ryegrass with oats in spring and while the oats are harvested in autumn, the ryegrass is allowed to grow until late in the following summer when the flowering heads have appeared and ripened.

Under suitable conditions of moisture and temperature the 'blind' seed fungus produces a Helotium-like apothecium, in which it has been reported a sexual phase occurs. Apothecia are formed at the time of flowering of the ryegrass and appear just above the soil surface, being produced from 'blind' seeds, lying on or slightly below the soil surface. The ascospores produced in the apothecia are liberated and are carried by air currents to the ryegrass florets where they germinate, attack the ovary and set up new infection. The formation of a pinkish conidial slime follows, the spores in which are dispersed to other florets by rain and probably to other plants by insects. Environmental conditions favourable for a heavy infection appear to be present when frequent rain occurs between the time of flowering and harvest.

As far as is known the disease is not systemic and although the most serious damage is confined to ryegrass, it has been shown by Wilson et al. (9) that the 'blind' seed fungus can also infect rye.

Neill and Hyde (6) also demonstrated the presence of

at least five other fungi connected with infected ryegrass plants and amongst them was one termed by these workers Lolium fungus No.2. Blind seed disease was originally associated with a fungus of the genus Pullularia which is Discomycete and a member of the family Helotiaceae. This organism was isolated from 'blind' seeds by Noble (7), but Wilson et al. (8) state that both this fungus and the true 'blind' seed fungus may be present on the same diseased seed and that both fungi are very similar. Muskett and Calvert (5) in arriving at the same conclusion observed that Pullularia sp. is not parasitic and is found on the glumes and the exterior of the caryopsis, while the 'blind' seed fungus occurs only on and in the caryopsis. These workers also found a difference in conidial size in the two fungi and state that whereas inoculation of ryegrass plants with the 'blind' seed fungus gave an infection of 10.6% on the resulting seed, inoculation with Pullularia conidia had no effect.

Muskett and Calvert (5) in Northern Ireland found apothecia developing on dead ryegrass seeds on turfs collected from the field. These apothecia were similar to those described by Neill and Hyde (6) in New Zealand and the ascospores produced from them gave rise to typical 'blind' seed infection. Glasscock (2) also reported similar apothecia in Kent and observed that the presence of the 'blind' seed fungus was the

cause of low seed germination. Gemmell (1), who found that the disease was more severe on late than on early strains of ryegrass, also showed from many samples of seed that the presence of conidial infection on the caryopsis of ryegrass seed could be closely correlated with the failure of the seeds to germinate. Wilson et al.(9), however, do not agree that this is the cause of low germination and instance cases where 'blind' seed apothecia were found on ryegrass seeds which gave rise to otherwise normal and healthy plants.

Most of the ryegrass grown for seed in Great Britain is cultivated in Northern Ireland. Ayrshire and Kent are the other two chief districts where the ryegrass crop is extensively grown. Seed from these and other areas has been found to be infected, in some cases very heavily with 'blind' seed disease which according to Wilson et al.(8) has been present in Britain since at least 1936. The severity of the damage which can be caused by the 'blind' seed fungus may be seen in the case of one particular strain of ryegrass which was grown in a district in Ayrshire (1). In 1939 the total crop of 3 cwt. per acre gave only 1¼ cwt. of cleaned seed, the loss being due to probably an early infection with the fungus and the resultant production of small and shrivelled seeds. Furthermore, in this 1 cwt. 50% of the seeds were infected with the 'blind' seed fungus which would doubtless interfere with their

germination.

Control of the Disease.

It was found by Gorman (3) in New Zealand that the best yields of pure germinating seed were obtained from crops which have produced a dense growth and especially when a pedigree strain of white clover was used in association with the ryegrass. The dense growth at an early stage appears to be important in checking the developmental stages of the fungus. This author also reported that hot water treatment of the seed and dusting with mercurial compounds both gave negative results.

When the following experiments were performed (1940) no methods of control had been established although some had been outlined, and much of the information recorded in the previous pages had not been published.

Experimental Methods and Data.

It will be remembered that the apothecia of the 'blind' seed fungus are generally formed on ungerminated ryegrass seeds which lie on or slightly buried in the soils, and appear at about the time of flowering of the plant. Accordingly, the method of control adopted was to attack the apothecia by means of chemicals.

Laboratory Experiments.

In order to study closely the effect of the substances employed, and incidentally to obtain information about the apothecia and the extent of their occurrence, turfs were dug from the ryegrass fields and kept in a hot greenhouse. It was considered that in this way apothecia would be produced earlier than in the field.

Six fields were visited at the beginning of May 1940 and from each field ten turfs, each one foot square, were taken. Four of the fields had been sown with ryegrass in the spring of 1938 and harvested in 1939, the seed produced having shown a high percentage infection. The two other fields had been sown in 1939 and were harvested in 1940, after these experiments had been concluded. The variety of ryegrass sown in all cases was S. 23, a late strain, shown to be severely attacked by the 'blind' seed fungus, when grown in Ayrshire (1).

The fields from which the turfs were taken are noted in Table I. with the corresponding degree of infection on the ryegrass crops.

The uncleaned crop from field C was 3 cwt. per acre but when the seed was cleaned the figure dropped to 1¼ cwt. per acre of which about 50% was infected. (1).

The turfs were laid on trays in a greenhouse and kept damp at a temperature of about 80°F. The grass was cut

Table I.

	Sowing	Farm	Infection on crop (%) Total	Severe	Slight
A	1938	Crookside, Monkton,	52	24	28
B	1938	Southside, Monkton,	48	32	16
C	1938	Newlands, Kilmaurs,	52	28	24
D	1938	Mid-Buiston, Kilmaurs,	60	32	28
E	1939	Newlands, Kilmaurs,	-	-	-
F	1939	Mid-Buiston, Kilmaurs,	-	-	-

closely to facilitate observation of any apothecia which might appear and it was decided to wait until their presence had been demonstrated before any application of chemicals was made. The turfs were examined regularly and after about one week several types of apothecia were observed on small pieces of oat stems and on decaying ryegrass stems. These were believed to be the fruiting bodies of saprophytic fungi of the genus Sclerotinia.

After about a fortnight, a few apothecia were discovered growing on ungerminated ryegrass seeds on two of the turfs of series A. A few days later, a careful examination gave the following result. (Table II.)

No correlation was apparent between the numbers of 'blind' seed apothecia and the percentage infection of the previous year's crop, for it might have been supposed that the seeds which fell when the crop was being harvested would have

Table II.

Extent of apothecial formation on turfs.

Field	Number of the 10 turfs showing apothecia on 'blind' seeds.	Number of other apothecia present
A	10 (some with 10 apo.)	many
B	1 (1 apo.)	very few
C	9 (one with 14 apo.)	many
D	1 (1 apo.)	few
E	1 (1 apo.)	many
F	0	few

indicated the amount of the disease present.

Measurements of the asci, ascospores and paraphyses of the 'blind' seed apothecia, together with measurements of the macroconidia from the caryopsis, were made, using an eye-piece micrometer. They are shown on Table III., compared with the corresponding figures given by Neill and Hyde (6).

Table III.

Comparative dimensions of 'blind' seed fungus.

	Length x breadth of ascus (μ)	Length x breadth of ascospore (μ)	Diameter of paraphysis (μ)	Length x breadth of macroconidia (μ)
Neill & Hyde,	75 x 5	7.5 x 3.5	1.75	17 x 3.5
Writer,	82 x 5	7.0 x 3.5	2.0	15 x 3.4

Application of Chemicals. It was interesting to note that field B. whose turfs showed only one 'blind' seed apothecium, had been limed at the rate of 17 cwt. per acre a few months previously, and pieces of lime could still be seen in the surface layers of soil, while field A. had received no lime and many apothecia were found. However, the other fields (except F.) had also received applications of lime, although not so recently. Nevertheless, it was decided to include lime as one of the experimental treatments.

The different substances used and the rates at which they were applied were as follows:-

Lime, ...	30 cwt./acre	i.e., 5/4 oz.per sq.ft.
Vitagreen, ...	3 cwt./acre	i.e., 1/8 oz.per sq.ft.
Calcium Cyanamide,	3 cwt./acre	i.e., 1/8 oz.per sq.ft.
Sulsol, ...	30 lb. /acre	i.e., 32 cc.1% suspn.per sq.ft.

The freshly ground and sieved shell lime which was used, and the Vitagreen - a proprietary compound, containing 3% inorganically combined mercury - were mixed with their own bulk of fine sand in order to obtain a more uniform application.

Two turfs from each field were subjected to each treatment, the controls being left untreated, and all the turfs randomised and kept in the same conditions of temperature and humidity in the greenhouse as before.

Effects of Treatments. After a few days the burning effect of cyanamide on the grass was very noticeable but none of the other treatments appeared to have affected the growth of the grass. Some days later, an examination was made for the presence of all types of parasitic and saprophytic apothecia, since it was reasonable to assume that substances having no effect on similar fruiting bodies would afford no control of the production of 'blind' seed apothecia. The examination was repeated after one week with the following results.

Only the turfs from two sources - namely Crookside and Newlands 1938 sowings - could provide significant results, since an abundant production of apothecia was originally evident in only these turfs. It was found that in the control turfs from these sources, 'blind' seed or other similar apothecia were present in all cases. After Sulsol treatment, these apothecia were still evident although only in 50% of the cases, but none were observed in the turfs to which other compounds had been applied.

From further examinations of the turfs, it was noted that Vitagreen had inhibited the formation of apothecia of all types for at least six weeks. A few of the Sclerotinia type of fruiting body were formed during this time after applications of lime and cyanamide.

The ryegrass on all these turfs was allowed to grow

on after the initial cutting and when the seed had set, samples were taken from the plants and examined. No sign of the presence of the disease was observed on any of the seeds, but this may have been due to the early cutting of the plants to facilitate the search for apothecia.

Field Experiments.

In view of the complete absence of experimental data which existed when these tests were carried out, regarding the control of 'blind' seed disease on a field scale, two field trials were undertaken. The object was to destroy the apothecia of the fungus or to inhibit their formation by chemical means, so as to remove the immediate source of infection. In this way the seed produced would be, if not entirely free from disease, much healthier than if no treatment had been applied. The most suitable time for application of the control substances was reckoned to be shortly before the time of flowering of the ryegrass.

Three plots, each 20 yds. by 40 yds., i.e., 1/6 acre in area, were laid down in two fields of ryegrass, variety S.23, which had been sown out with oats, as is the usual practice, in the spring of 1939. One field was at Newlands Farm, Kilmaurs, Ayrshire, and the other at Crookside Farm, Monkton, Ayrshire. The substances applied to the plots were ground shell lime at

the rate of 30 cwt. per acre, and Vitagreen at the rate of 3 cwt. per acre, while the middle plot in each field was untreated. The applications of Vitagreen (½ cwt.per plot) and lime (5 cwt.per plot) were all made between June 5-8, 1940, when the flowering heads of the ryegrass plants had appeared and the grass was about 1½ ft.and 2 ft.high in the respective fields.

The rather lengthy period of dry weather which followed the application of the compounds was not conducive to their rapid penetration to the surface of the soil, but the grass itself did not appear to be damaged in any way.

At the end of July, when the seed was harvested, samples of the ryegrass heads were taken from all parts of the treated and control plots and left to dry in the laboratory. The dried seeds were then extracted and examined for the presence of conidial infection on the caryopsis, but no sign of disease was found on any of the seeds examined from treated or control plots in either of the fields, and no differences were observed in germination tests with the seeds.

Discussion.

By cutting turfs from fields which were known to have grown ryegrass infected with 'blind' seed disease, and keeping them in warm and damp conditions, it was possible to

demonstrate the occurrence of apothecia of the 'blind' seed fungus on 'blind' seeds, which lay on or slightly buried in the soil. Measurements of asci, ascospores and paraphyses formed in these apothecia, when found in Ayrshire, as well as those of the macroconidia formed on the surface of the caryopses were shown to be similar to the corresponding figures given by Neill and Hyde (6) from New Zealand.

The extent of apothecial production present in the turf samples could not be correlated with the amount of disease present on the previous crop of ryegrass seed, grown in the fields from which the turfs were taken. It is difficult to account for this, but it may have been due, in one case at least, to the application of lime, or else early harvesting may have been carried out so that few of the diseased seeds had fallen back to the soil.

From the control experiments, conducted in the laboratory, it may be concluded that, since blind seed apothecia were observed within two weeks of the treatment of the turfs with Sulsol, this compound, at the rate of application employed, will not give the desired control of 'blind' seed disease. Also, although cyanamide destroys the apothecia or temporarily inhibits their formation, it is injurious to the grass. Lime was shown to inhibit the production of apothecia, but the most efficient of the substances used was Vitagreen,

the toxic principle of which is mercury.

It was unfortunate from an experimental point of view that there should have been such a lack of infection in the field trials. This freedom from 'blind' seed disease was, however, a general feature of the 1940 crop of ryegrass in Ayrshire, as was shown by examination of many other samples, and was probably occasioned by the absence of heavy rain during the period between the flowering of the ryegrass and harvest. The absence of conclusive results from the field trials was the more unfortunate, since it was found impossible to repeat the experiments or to continue investigations on the disease.

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