ECOLOGY AND VIRUS-VECTOR RELATIONSHIPS OF SOME PLANT PARASITIC NEMATODES

by

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ABSTRACT

The ecology and virus-vector relationships of *Xiphinema diversicaudatum* with the hop strain of arabis mosaic virus (AMV-H) and of *Longidorus caespiticola* and *Longidorus leptocephalus* with the 'English' strain of raspberry ringspot virus (RRV-E) were studied in the laboratory and in the field. Hop was a poor host for *X. diversicaudatum* which did not aggregate on roots. Weeds common in hop fields proved more suitable than hop plants for the nematode's multiplication. In field hops, all three nematode species occurred to a depth of 90 cm with peak populations of *X. diversicaudatum* at 11-19 cm and of *L. caespiticola* and *L. leptocephalus* at 26-34 cm.

Using small containers, hand-picked *X. diversicaudatum*, reared on nettlehead-diseased or other AMV-infected hops, transmitted AMV-H efficiently to hops, some herbaceous indicators and hop-field weeds. Viruses causing nettlehead and line-pattern symptoms were transmitted by nematodes for the first time. AMV-H persisted in starved *X. diversicaudatum* larvae for 29 weeks and for 36 weeks in adults. Temperatures profoundly influenced symptom expression of AMV-H in herbaceous indicators and 14-18°C was optimal. AMV-H was not detected serologically in hop leaves until at least 27 weeks after nematode inoculation of roots. *Petunia hybrida*, *Chenopodium murale*, *Cirsium arvense*, *Senecio vulgaris*, *Atriplex patula* and *Chenopodium album* were found to be hosts of AMV-H; the virus was seed-borne in the last two species and also in *Chenopodium quinoa*.

RRV-E was transmitted by L. caespiticola and L. leptocephalus. Efficient transmission of RRV-E by X. diversicaudatum was demonstrated and the virus persisted in starved nematodes for at least 26 days. From a mixed infection of RRV-E and AMV-T (arabis mosaic virus, type strain) X. diversicaudatum transmitted both viruses simultaneously, but AMV-T more readily, whereas L. caespiticola transmitted with equal efficiency.

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PART I

ECOLOGY AND VIRUS-VECTOR RELATIONSHIPS OF *XIPHINEMA DIVERSICAUDATUM* (MICOL.) AND THE HOP STRAIN OF ARABIS MOSAIC VIRUS

INTRODUCTION

Hop, *Humulus lupulus* L., is affected by a number of virus diseases. Among them, nettlehead, which affects all varieties but is most severe in Fuggle, appears to be the oldest and probably the most serious. This disease occurs in all hop growing areas in England and is reported to be serious in Tasmania (Johnstone, 1965). Perhaps the first recorded account of the occurrence of nettlehead in England was made by Reynolde Scot in 1574 in a brief description cited by Legg (1964) thus: 'The Hoppe that lykes not his entertainment, namely his seat, his grounde, his keeper, his dunge, or the manner of his setting, etc., appeareth at the first out of the grounde greener and small in stalk, thicke and roughe in leaves verye lyke unto a nettle'. In 1870, Charles Whitehead described nettlehead-like rotting symptoms of hop roots in old gardens in Kent, and in 1894, one hundred farmers complained of the seriousness of nettlehead in a meeting at Wye College (Keyworth and Paine, 1950). It was Percival (1895) who first described nettlehead disease and ascribed it to the attacks of the nematode *Heterodera schachtii* Schmidt. Duffield (1925), however, disproved this when he showed that there were no significant differences between the number of nematodes on the roots of healthy hops and those with nettlehead disease. Salmon and

Ware (1930) and Ware (1939) obtained evidence that nettlehead was graft-transmissible. This observation was confirmed by Keyworth (1941) and Keyworth and Davies (1946) who concluded that nettlehead was transmissible by inarching and that it was probably a virus disease. Early attempts to transmit nettlehead through the soil were unsuccessful (Keyworth and Davies, 1946; Anon, 1951, 1958; Legg and Ormerod, 1959). However, further work was stimulated by the discovery that Xiphinema diversicaudatum (Micol.) was the vector of a strawberry isolate of the type strain or arabis mosaic virus (Jha and Posnette, 1959). Legg (1964) transmitted nettlehead to Early Prolific hops by grafting and by manual inoculation with leaf and root extracts of diseased hops and with concentrated, partially purified preparations from nettlehead-diseased hops. The presence and identity of a strain of arabis mosaic virus (AMV-H) in nettlehead-affected and in symptomless hop plants was shown by Bock (1966) by electron microscopy and serology. Consistent occurrence of numerous X. diversicaudatum was reported in hop sites in Kent where nettlehead rapidly became prevalent in monthly-planted AMV-free setts and in an area at Rosemaund Experimental Husbandry Farm where severe split leaf blotch had occurred within two years of replanting with virus-tested stocks (Bock, 1966; Anon, 1967). In common with nettlehead-diseased hops, AMV-H was also demonstrated in plants showing 'bare bine' and severe split leaf blotch symptoms by gel-diffusion serology (Anon, 1969; Thresh and Ormerod, 1970). A brief description of these diseases follows:

Split leaf blotch.- This disease occurs only in the variety Fuggle. The first symptoms during the early growing season in March or April are referred to as 'bare bine' or 'spidery hop' (Wilkinson, 1946). The stems are few and weak in growth, some being small and stunted compared with the numerous growths and bushy appearance of healthy plants. Bines of affected plants are often dark green or reddish in colour and affected plants usually occur in small groups or rows. These symptoms are lost after the bines are trained up the strings and superfluous shoots are removed.

Later in the season, symptoms appear on young leaves. Irregular areas of the lamina turn pale yellow and appear transparent and oily. The uneven growth results in a distorted leaf and eventually the affected patches of tissue die and tear or fall away, leaving irregular holes and splits (Keyworth, 1951).

Nettlehead.- Affected bines are rigid and with short internodes, lose their climbing power and fall away from the strings, giving a characteristic dwarf appearance, become very brittle and of a dull-yellowish brown colour. The basal lobes of newly formed leaves are small or absent, the leaves stand out stiffly with their edges curled upwards resembling those of a nettle, and sometimes develop enations. This disease does not kill the plant but renders it weaker year by year (Keyworth and Davies, 1946).

The present study, conducted at East Malling Research Station from October, 1968 to May, 1971, aimed to investigate the ecology and virus-vector relationships of Xiphinema diversicaudatum and the hop strain of arabis mosaic virus (AMV-H) in nettlehead disease.

MATERIALS AND METHODS

The following biological materials were used throughout the study:

Xiphinema diversicaudatum (Micol.):

a) Non-infective - from 'woodland soil' at High Halstow, Kent, containing nematodes of which about 98% were *X. diversicaudatum*; referred to subsequently as 'woodland *Xiphinema*'.

b) Infective:

(i) Woodland *Xiphinema* raised on Fuggle hop plants that became infected with AMV-H when planted for one growing season in a site where split leaf blotch occurs at Slade Yard, Rosemaund Experimental Husbandry Farm, Herefordshire. However, the actual infector plants never showed the disease when grown in wooden boxes (20 cm x 30 cm x 45 cm) for at least four months; this isolate is referred to in the text as 'Slade soil box *Xiphinema*'.

(ii) From soil around roots of hops (Bramling Cross) with severe nettlehead disease at Nevergood Farm, Horsmonden, Kent; referred to as 'Field nettlehead *Xiphinema*'.

(iii) Woodland *Xiphinema* raised on nettlehead-diseased Fuggle hops from Grigg Farm, Headcorn, Kent, grown in boxes as in (i) and referred to as 'Nettlehead soil box *Xiphinema*'.

AMV isolates:

a) AMV-H (S) - from hop source plants mentioned in b(i) above.

b) AMV-H (N) - from hop source plants mentioned in b(ii) above.

c) AMV-T - from strawberry.

Ecology of Xiphinema diversicaudatum on hop

In root observation boxes.- Root observation boxes (15 cm wide x 45 cm long x 45 cm deep) provided with detachable glass frames on two sides and containing 1.1 cu.ft of soil were used (Fig. 1). The inner sides of the box were lined with clear 'Melanex' polyester sheet to hold the soil in place when the glass frames were removed during sampling. A removable lid outside the glass frame shielded the roots from light. The woodland soil used was screened through 3 cm mesh and mixed thoroughly before placing it in the boxes. The initial number of the different growth stages of the nematode was assessed from samples taken at random. One lot from the same batch of soil, placed in a 'polythene' (polyethylene) bag and kept at 3°C, was used to replace the samples taken during the experiment. Root-free areas in each box, referred to in the text as 'soil cylinders', were maintained by putting part of the soil in rigid polythene tubes (7 cm diameter x 15 cm long), each provided with three equidistant side cut-outs (2.5 x 9 cm), on which and on both ends were 'soldered' 50 micron mesh nylon bolting cloth which the nematodes, but not the hop roots, could penetrate. Four soil cylinders were buried horizontally in each box with their ends touching the 'Melanex' sheet on both sides (Fig. 1). A hop seedling or sett was planted in the centre



Fig. 1. Root observation box used in ecological studies of Xiphinema diversicaudatum in hop; the ends of four root-free 'soil cylinders' buried horizontally are visible through the glass panels.

of each box, except in the controls, which contained soil only. The soil surface was covered with black polythene sheet to minimize drying and each box was watered similarly.

Soil samples on and around selected primary roots and along mats of lateral roots were compared with adjacent sites 8 cm from roots. Samples were taken contiguously with a 1 cm diameter semi-cylindrical brass core-sampler driven horizontally 1 cm deep. However, a 2.5 cm diameter core-sampler, driven 7.5 cm deep, was used to take the samples inside and outside the soil cylinders as well as those along the vertical and horizontal axes of the root box. Samples inside the soil cylinders were taken at their centres and those outside, exposed to hop roots, were taken horizontally, 12 cm distant from the centre of the adjoining cylinder. Each sample was washed into a glass tumbler and nematodes were extracted by a modified Cobb's decanting and sieving dish technique, henceforward referred to as Flegg's technique (Flegg, 1967); adults and larval stages were counted separately.

Under field conditions.- The site selected for this study was a 3-acre hop field at Horsmonden, Kent. This planting (Bramling Cross and W.G.V.) has been badly affected with nettlehead disease and previous samplings in the area yielded appreciable numbers of *X. diversicaudatum*.

Soil samples were taken, at sites randomized throughout the field, between and within hop rows at various depths and distances from the plant in September, 1969. Sampling between rows was done by first digging a trench 15 cm from the hop hill, about 40 cm wide, 90 cm deep and 1 m long.

Then twin-core samples were taken with a trowel horizontally at 11-19, 26-34, 41-49, 56-64 and 71-79 cm depths at 0, 45 and 90 cm distances from the plant. Each sample was gently crumbled, mixed and a 200 ml subsample was processed for nematodes by Flegg's technique. After a 20-hour extraction time, the nematodes were collected and the adults and larval stages were counted separately.

When sampling between hills, holes about 30 cm square and 15 cm deep were first dug with a spade at 15 cm and 90 cm distances from the hop plants (i.e. midway between two hop hills). Twin-core samples were then taken with a trowel at 15-30 cm deep in the holes and a 200 ml subsample from each was processed as in the sampling between rows.

The data, and those obtained from the root observation boxes, were transformed into square roots and analyzed statistically.

Survival and multiplication of *Xiphinema diversicaudatum* on weeds and on virus indicators

Seeds of weeds common in hop fields and of some virus indicators used in nematode transmission tests were sown in steamed soil on dates arranged so that all species germinated at roughly the same time. One seedling of each test plant was transplanted at the first true-leaf stage into a 90 ml clay pot, with drain hole plugged with plaster of Paris, containing a 3:1 steamed soil-sand mixture. The eight replicates of each plant species, including the unplanted control, were randomized in blocks with the pots partly embedded in moist sand to reduce fluctuations in the

environment. Two weeks after transplanting, each plant was infested, via a hole dug into the root zone, with 20 males and 30 females of X. diversicaudatum that had been starved for eight months. Ten weeks after infestation, the nematodes were extracted and males, females, different larval stages and uterine eggs counted separately. The roots were examined for galls and representative samples were stained in acid fuchsin-lactophenol and photographed.

Virus-vector relationships of AMV-H and Xiphinema diversicaudatum

Nematodes.- All infective nematodes used in studies on virus-vector relations were extracted by Flegg's technique, including those raised on shoot-tip cuttings of nettlehead-diseased hops rooted in polythene ice-cube moulds. Often, hand-picking and inoculation commenced after 3 hours extraction through nylon screens in dishes or at most overnight, thereby ensuring that all the nematodes were active. Moulting nematodes were excluded.

Virus transmission by nematodes.- All nematodes used in transmission tests were hand-picked under the binocular microscope with the aid of a finely pointed bamboo splinter. The nematodes were collected in wells of perspex cavity blocks and then scooped into small containers consisting of plastic egg cups (20 ml) or small clay pots (20 ml, with the drain hole plugged with plaster of Paris), each containing 1 ml steamed soil-sand mixture; just enough water was used to wash off the ~~inoculated~~ nematodes. Chenopodium quinoa and Fuggle hops used as bait plants were inoculated at

cotyledon to first true-leaf stage. The roots were first carefully washed, placed over the nematodes, covered with about 5 ml of steamed soil-sand mixture and watered from a wash bottle or pipette. The egg cups or clay pots were partly embedded in moist sand to reduce environmental fluctuations. After 4-5 weeks, the roots of the bait plants were washed free of soil and nematodes, ground with 'Superfloss Celite' (Johns-Manville Ltd.) and inoculated on leaves of C. amaranticolor indicators at the 6-leaf stage. All inoculated indicator plants were incubated in a growth chamber at 14-18°C. The presence of virus in test plants showing systemic symptoms was confirmed by gel-diffusion serology with expressed sap and a 1:40 dilution of antiserum to the type strain of arabis mosaic virus, isolated from strawberry.

Virus transmission through seed.- Seeds were collected from individual plants infected with AMV-H at the seedling stage by X. diversicaudatum or by sap-inoculation. These were sown two weeks later, in steamed soil in the greenhouse. Two weeks after sowing, when the roots were about 3 cm long, 30 seedlings from each plant were transplanted singly into polythene ice-cube moulds (10 ml), provided with fine drain holes and a piece of nylon bolting cloth at the bottom to prevent roots from protruding. The ice-cube moulds were embedded to the brim in moist sand to minimize watering until each plant was indexed for virus. This was done 6 weeks after sowing the seeds, by inoculating the sap to C. amaranticolor. The presence and identity of virus in indicator plants showing systemic symptoms was confirmed by gel-diffusion serology.

RESULTS

ECOLOGY OF XIPHINEMA DIVERSICAUDATUM ON HOP

Studies in root observation boxes

Nematode distribution in relation to primary roots.- Commencing in May, 4-5 weeks after planting the hops, white, fast-growing primary roots were observed through the sides of the root box. The daily increments of this type of root measured over 7-14 days averaged 1 cm in the 1969 trial, when seedling hops were used, and 1.5 cm in the 1970 trial, when rooted cuttings were planted. Following the procedure of Pitcher (1967) for Trichodorus viruliferus, only three samples were taken in the rhizosphere during the 1969 trial. However, after considering the faster growth rate of hop roots compared with apple roots and the bigger size of X. diversicaudatum compared with Trichodorus, the 1 cm diameter contiguous sampling during the 1970 trial was extended back to 9 cm from the tip, where lateral roots were beginning to form.

Examination of excised root tips under the microscope showed neither nematodes nor any apparent cell discolouration that may have resulted from nematode feeding. The mean number of X. diversicaudatum obtained from the rhizosphere samples did not differ significantly along the root from the tip throughout the 9 cm length (Table 1). Similarly, the mean number of nematodes in the non-rhizosphere soil obtained in front, at left and at right of each root tip did not differ significantly from that in the rhizosphere area. Similar results were obtained in both trials.

Table 1. Distribution of *Xiphinema diversicaudatum* in relation to primary roots of hops grown in observation boxes

Distance from root tip (cm)	Mean number per 1 ml soil			
	Rhizosphere		Non-rhizosphere	
	1969*	1970†	1969*	1970†
1.5 Front			2.06	3.41
1.5 Left			2.96	2.92
1.5 Right			1.79	2.10
0	2.84	4.24		
1	1.50	3.24		
2	2.69	2.88		
3		2.92		
4		4.24		
5		3.80		
6		3.12		
7		2.96		
8		2.61		
9		3.67		

*S.E. = 0.212 (Data shown are the means of 5 root samples in $\sqrt{x + 1}$ transformation)

†S.E. = 0.196 (Data shown are the means of 12 root samples in $\sqrt{x + 1}$ transformation)

Nematode numbers in sites with and without roots.- As no differences were found between the nematode populations in the rhizosphere and in the non-rhizosphere, it was thought that perhaps because of its large size, X. diversicaudatum wandered away from the roots after feeding. The results of contiguous sampling taken with 1 cm diameter core sampler on (a) a mat of lateral roots formed between two primary roots, (b) tips of lateral roots arising from one primary root, and (c) areas 8 cm from roots, gave no significant differences between the number of X. diversicaudatum in any of these sites (Table 2). Similarly, the number of nematodes in samples within each site did not differ significantly. These results corroborate those in Table 1 which showed that X. diversicaudatum, unlike Trichodorus viruliferus on apple roots (Pitcher, 1967), does not aggregate on hop roots.

Movement of nematodes from root-free soil cylinders towards roots.- During the 1969 test, with four replicates, samples were taken in July, August and October, i.e., 3, 4, and 6 months after planting the hops, respectively. In samples obtained in July and in August, the number of X. diversicaudatum inside the root-free soil cylinders did not differ significantly from the counts outside the soil cylinders exposed to hop roots (Table 3). In the October samplings, however, the number of nematodes outside the soil cylinders were significantly more than the number inside the soil cylinders. The experiment was repeated in 1970 using soil with a higher population of X. diversicaudatum and eight replicates. Control boxes with soil only were also provided. The results obtained in the root boxes with hops

Table 2. Number of *Xiphinema diversicaudatum* per 1 ml contiguous soil samples in root observation boxes containing hops

Location	Mean* (back-transformed)
Along mat of lateral roots between two primary roots	2.96
At tips of lateral roots arising from one primary root	3.16
In areas 8 cm from hop roots	2.80

*Three replicates of 10 samples each; data analyzed as $\sqrt{x + 1}$; S.E. of transformed mean = 0.103.

Table 3. Number of Xiphinema diversicaudatum inside and outside 'soil cylinders' (SC) in root observation boxes with and without hops[+]

Samples taken	1969	1970	
	With hops	With hops	Without hops
July			
Inside SC	39.7 (5.99)	129.5 (11.297)	105.9 (10.248)
	* 0.520	* 0.339	* 0.479
Outside SC	42.3 (6.32)	95.0 (9.712)	111.5 (10.515)
October			
Inside SC	14.5 (3.72)	56.7 (7.405)	107.5 (10.274)
	* 0.231	* 0.339	* 0.479
Outside SC	37.5 (5.96)	113.9 (10.465)	98.9 (9.888)

[+]Respectively, means of 4 and 8 replicates for 1969 and 1970 trials; ( ), means of data in $\sqrt{x + 1}$ transformations; * standard errors of transformed data.

were very similar to those recorded in the 1969 experiment, where the density of nematodes in October outside the soil cylinders and exposed to the hop roots was twice that inside the soil cylinders. In contrast, in the control boxes without hops, there were no significant differences between the number of nematodes inside and outside the soil cylinders in either the July or October samplings, suggesting that, in the absence of an attractant such as food (root), there was either no movement, or a 2-way movement through the mesh cut-outs in the soil cylinders. The results of these two experiments indicate that the nematodes in the root-free soil cylinders moved towards the hop roots but that it took them more than 4 months to travel a distance of about 10 cm. The significant reduction in the number of nematodes in October from the counts in July which occurred in both trials in the root boxes with hops, but not in the control, suggests that hop is either not a suitable host or that it is slightly toxic to X. diversicaudatum.

Counts of nematodes in the upper soil cylinders placed 12 cm from the surface and adjacent soil exposed to hop roots were compared with the numbers obtained in samples inside and outside the lower soil cylinders placed 33 cm from the surface. The results in both experiments showed that the density of X. diversicaudatum in the lower half of the root box was significantly higher than in the upper half, indicating that the nematodes tended to move downwards with the roots. This significant change in number was not detectable in July, but was apparent in August, 4 months after planting the hops.

Population structure.- To determine the effect of hops on the population structure of X. diversicaudatum, the number of males, females and the different larval stages was assessed in soil samples taken in April, when the hops were planted in root observation boxes, and in July and October, 3 and 6 months later. Control samples were taken from boxes without hops.

The percentage of adults (AD) and each of the different larval stages (L1, L2, L3, L4) in samples from root boxes with and without hops are shown in Table 4. Compared with the initial population in April, the population of L1 and L2 in July in root boxes without hops each decreased by 5%. Simultaneously, there was corresponding increase in L3 and L4 of 4% each and in adults of 3%. These results clearly indicate that moulting occurred in the absence of host plants and with 1% mortality between moults. No indication of moulting occurred after 6 months, suggesting that limited moulting occurred only within 3 months of the start of starvation. There was no further indication of moulting between 3 and 6 months from the onset of starvation, respectively, between July and October.

In root boxes with hops, however, the proportion of L1 and L2 in July decreased by 11% and 6% each. At the same time, there was an increase in L3, L4 and adults by 2%, 8% and 7%, respectively. These results indicate that a higher percentage of nematodes moulted, compared with those in the unplanted control. The big reduction in the proportion of L1, which was not matchod by an increase in the subsequent stages, further suggests that, if hop contains any toxic

Table 4. Changes in population structure (%) of *Xiphinema diversicaudatum* in root observation boxes with and without hops

Stage	Initial population (April)+	% Composition after 3 months (July)					% Composition after 6 months (October)				
		L1	L2	L3	L4	AD	L1	L2	L3	L4	AD
L1	16.3	5.2*					1.0*				
		10.6**					10.8**				
L2	17.8		11.6*					6.4*			
			12.6**					9.7**			
L3	19.1			20.8*					24.0*		
				22.8**					21.8**		
L4	12.2				20.0*					27.3*	
					16.2**					20.5**	
AD	34.7					42.4*					41.4*
						37.8**					38.0*

+Mean of 6 x 200 ml samples.

*With hops; mean of 32 x 30 ml samples.

**Without hops; mean of 32 x 30 ml samples.

substance, it is the L1 stage that is most vulnerable. After 6 months, only 1% L1 remained and moulting of L2, L3 and L4 became more marked.

In root boxes with and those without hops in the July and October samplings there were, in all cases, approximately 5% more females than males.

Vertical and horizontal distribution.- The distribution of nematode vectors in the soil is important not only in establishing their role in the etiology of a plant disease but also in relation to their control by chemical treatments. The following experiments report the results of studies on the vertical and horizontal distribution of X. diversicaudatum in soil boxes planted with hops.

Data obtained 6 months after planting the hops (Figs. 2 and 3) show that the nematodes were more numerous from 10 cm downwards and fewer at sites closer to the surface and to the sides of the root observation box. While the distribution of nematodes in the root boxes with hop appear to coincide with areas of maximum hop root density (Fig. 4), the nematode distribution in boxes without hops followed a similar pattern, suggesting that other factors besides root concentration, probably temperature and moisture, were involved. Starting with thoroughly mixed soil, marked changes in nematode density were detectable 6 months later, as shown by contiguous 2.5 cm diameter samples taken vertically and horizontally along the central axes of root observation boxes with and without hops. In general, there was a higher fluctuation (0-50) amongst the vertical samples taken from surface to bottom than amongst the horizontal samples (1-34) taken 22 cm

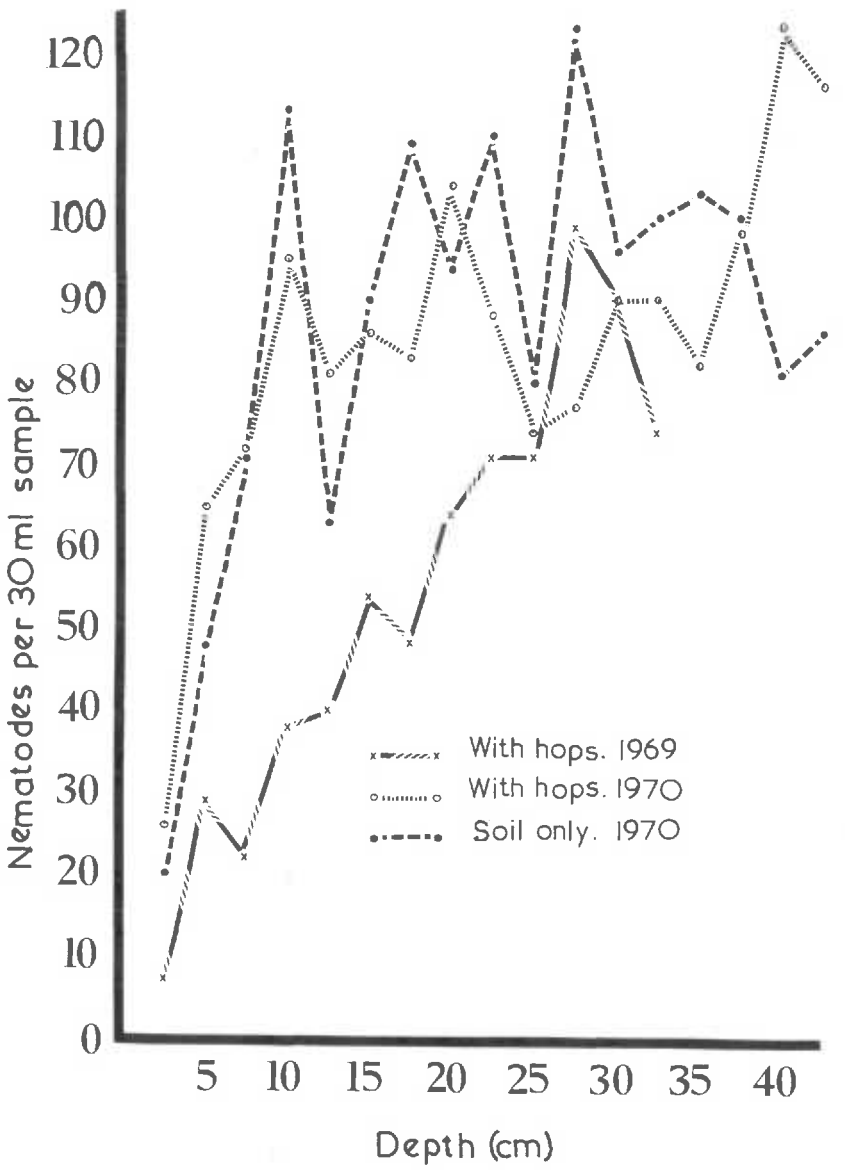


Fig. 2. Distribution of Xiphinema diversicaudatum in contiguous 2.5 cm diameter samples along vertical axis of root observation boxes with hops or with soil only.

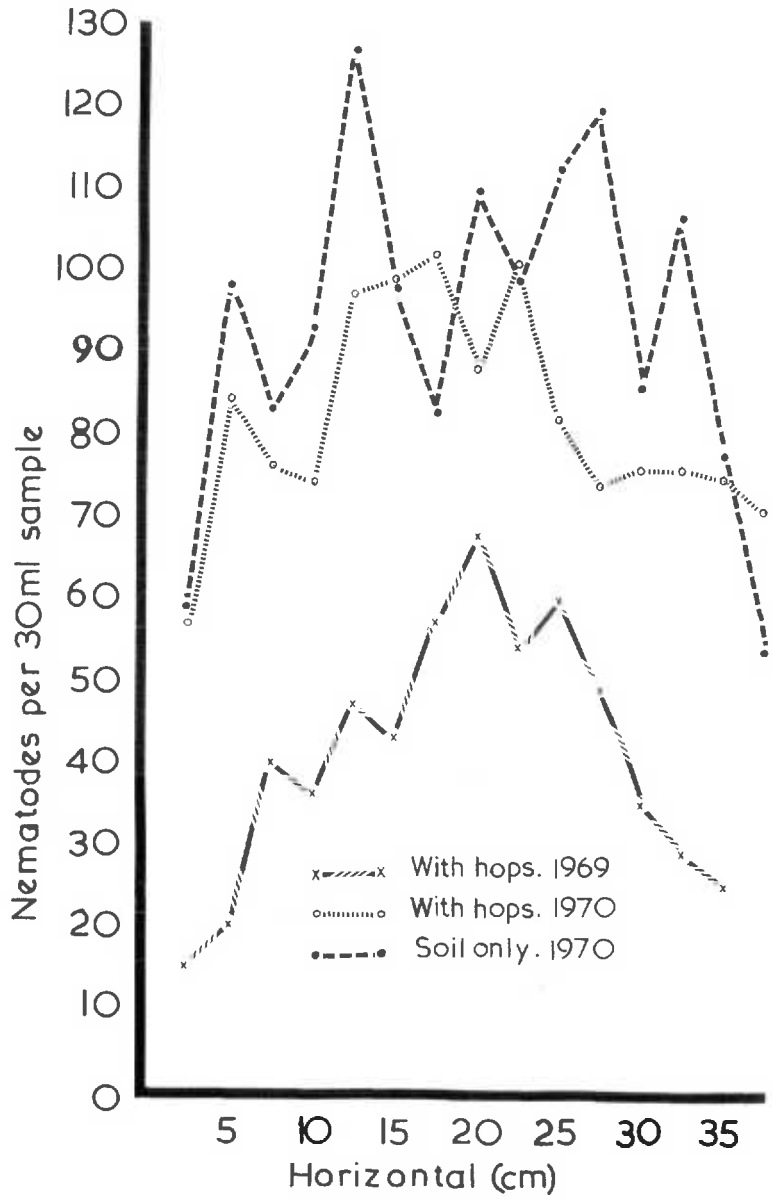


Fig. 3. Distribution of Xiphinema diversicaudatum in contiguous 2.5 cm diameter samples at 22 cm depth across root observation boxes with or without hops.

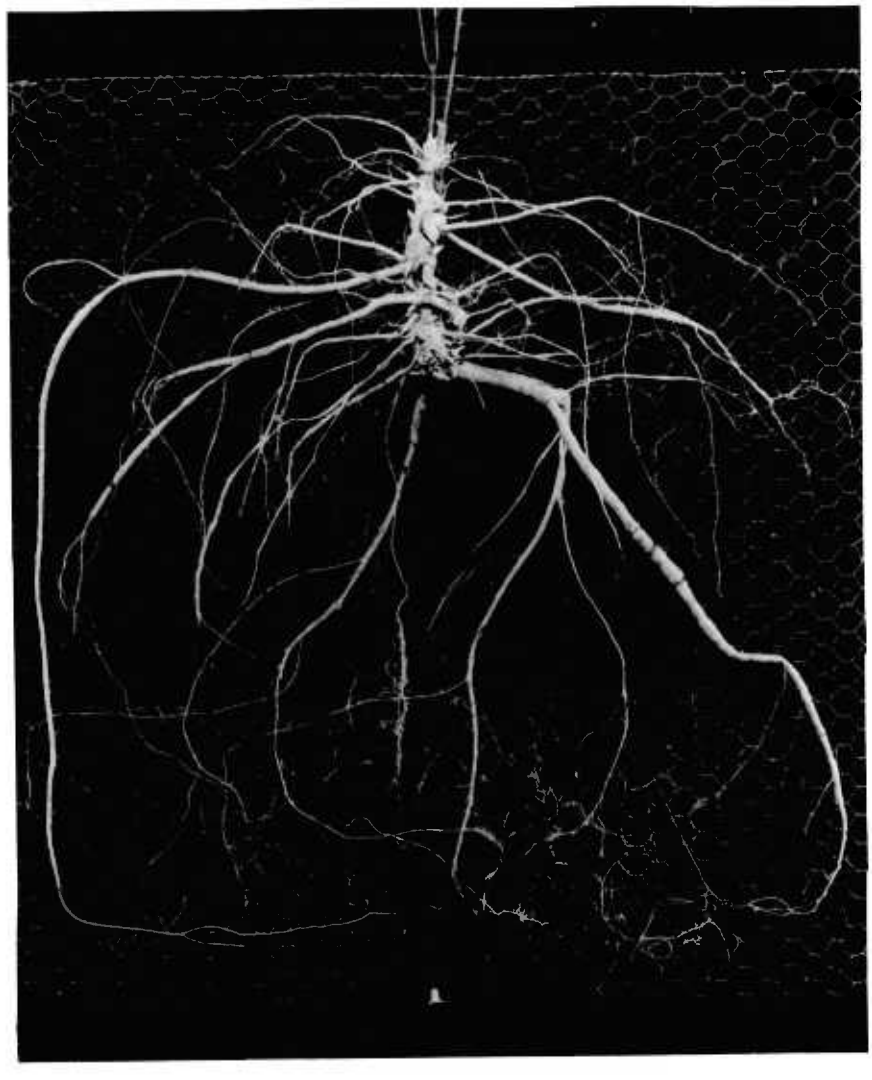


Fig. 4. Distribution of roots of hop grown in an observation box.

from the surface. Similarly, a higher fluctuation occurred amongst samples in boxes with soil only than in boxes planted to hops.

A remarkable redistribution of adults (AD) and the larval stages L2, L3, L4, in relation to depth, was obtained in the root boxes during the 1969 test (Fig. 5). Except for the L1s, which were very few, the density of L2s and L3s was highest at 5-15 cm from the surface and then gradually decreased with depth. In contrast, the density of adults and L4s increased with depth down to 40 cm. During the test in 1970, however, the number of L1, L2, L3 and L4 was highest at depths of 10-37 cm; results obtained with adults were as in the 1969 test.

Field Studies

Vertical and horizontal distribution.- The fluctuations in the number of X. diversicaudatum between 7 cm diameter contiguous samples taken at 15-30 cm deep in three parallel transects across the row of nettlehead-affected hops are given in Fig. 6. In the transect cut through the hop plant, there was very little fluctuation within 30 cm of the hop, but considerable fluctuation beyond. The transect cut at 30 cm from the hop gave the least fluctuation and the lowest nematode counts, probably due to partial exposure to high temperature when the transect at 60 cm was cut a week earlier. This test further showed the decreaso in numbers of X. diversicaudatum with distance from the hop plant.

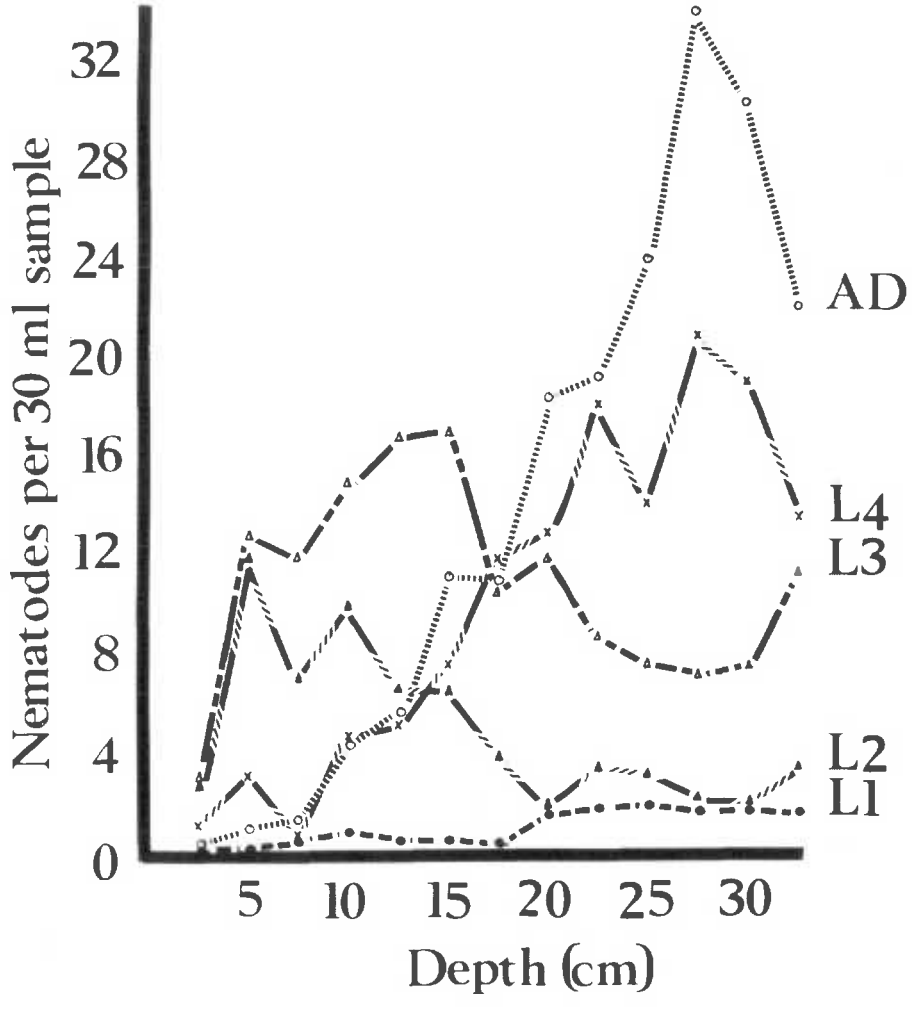


Fig. 5. Effect of depth on the population structure of Xiphinema diversicaudatum in root observation boxes after 6 months growth of hops. 1969.

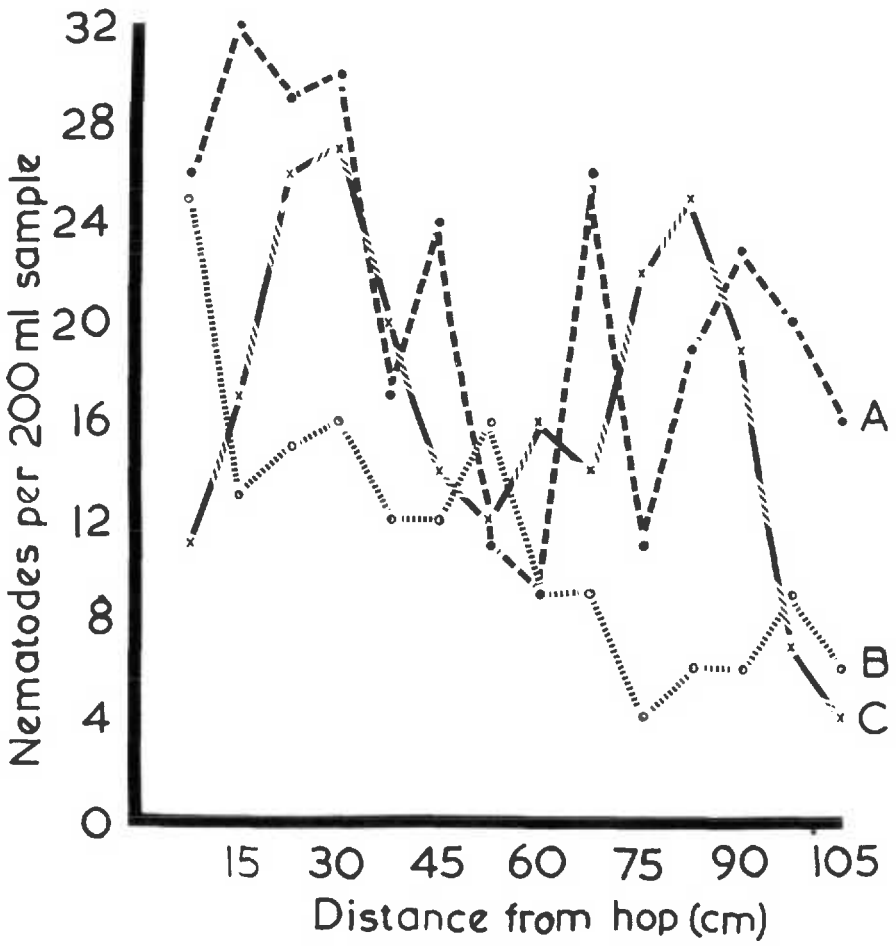


Fig. 6. Number of Xiphinema diversicaudatum in contiguous samples, 7.5 cm diameter, taken at 15-30 cm deep in parallel transects across the row (A) through hop plant, (B) 30 cm from hop plant, and (C) 60 cm from hop plant. (Horsmonden, Kent, 1969).

In samples taken between rows of field-grown hops, X. diversicaudatum were found at all depths from 11-79 cm and at the crown of the hop plant and 45 and 90 cm away (Table 5). However, they were most abundant at 11-19 cm deep and then decreased with depth and with distance. Using the basic data in Table 5, the totals of adults and the different larval stages in relation to depth are presented as percentages in Table 6. These results agree with the findings of Taylor and Thomas (1968) who reported that the ratio between the number of females and juveniles under Malling Jewel raspberry was similar at all depths. Based on the total counts of females and larvae in Table 5, the reproductive potential was two larvae per female.

The results of sampling between hills, given in Table 7, show that the density of X. diversicaudatum at 15 cm from the hop plants was significantly higher than at 90 cm, i.e. midway between two hop hills, thereby supporting the results given in Fig. 6 and in Table 5.

Laboratory Studies

Hop-field weeds and virus indicators as hosts of X. diversicaudatum.- Measured in terms of survival and reproductive potential of the nematode inoculum, the results of an experiment conducted in 90 ml clay pots from May-July, 1970 showed that all species of hop-field weeds and of virus indicators tested, except Cirsium arvense and Atriplex patula, were more suitable hosts of X. diversicaudatum than hops (Table 8). Similar results were obtained in March-May, 1969 in a preliminary test conducted in polyethylene

Table 5. Vertical and horizontal distribution of *Xiphinema diversicaudatum* in soil under hop plants Horsmonden, Kent, September, 1969*

Depth (cm)	Distance from hop (cm)			Mean
	0	45	90	
11-19	42.8 (5.8)	23.5 (4.4)	14.3 (3.5)	(4.6)
26-34	9.3 (3.1)	5.6 (2.5)	2.3 (1.7)	(2.4)
41-49	5.5 (2.5)	1.7 (1.6)	1.8 (1.6)	(1.9)
56-64	3.8 (2.0)	1.3 (1.5)	1.0 (1.5)	(1.6)
71-79	1.1 (1.3)	0.3 (1.2)	0.1 (1.1)	(1.2)
Mean	(2.9)	(2.2)	(1.9)	

*Mean of 12 x 200 ml samples in six replicates; ( ), means of data in $\sqrt{x + 1}$ transformation.

S.E. of transformed means are: depth -0.501

distance -0.289

Table 6. Population structure (%) of *Xiphinema diversicaudatum* at different depths in hop soil, Horsmonden, Kent, September, 1969*

Depth (cm)	Growth stage						Totals
	♂	♀	L1	L2	L3	L4	
11-19	15.3	18.8	17.8	3.7	5.7	9.1	70.3
26-34	4.8	4.3	2.7	0.5	0.8	2.0	15.1
41-49	3.4	1.8	1.5	0.3	0.1	0.8	7.9
56-64	1.5	1.3	1.3	0.3	0.3	0.6	5.4
71-79	0.1	0.3	0.7	0.0	0.1	0.1	1.3
Totals	25.1	26.4	24.0	4.9	7.0	12.6	100

*Based on total count in 180 x 200 ml samples taken at 0, 45 and 90 cm from hop plant.

Table 7. Effect of distance from hop plants in the row on the number of Xiphinema diversicaudatum at 15-30 cm deep, Horsmonden, Kent, September, 1969

Distance from plant (cm)	No. per 200 ml soil* ($\sqrt{x+1}$) ± S.E.
15	4.00 (± 0.170)
90	3.36 (± 0.241)

*Mean of 70 and 35 samples for the 15 and 90 cm distance, respectively, in five replicates.

Table 8. Survival and multiplication of *Xiphinema diversicaudatum* on weeds common in hop fields and on some virus indicators*

Host species	Nematodes recovered per pot after 10 weeks			Mean larvae per surviving female
	Inoculum		Eggs & Larvae†	
	♂	♀		
Stellaria media (L.) Vill.	13	21	131	6
Poa annua L.	16	22	56	3
Senecio vulgaris L.**	14	19	59	3
Sonchus oleraceus L.	15	19	37	2
Anthemis cotula L.	18	24	9	<1
Rumex obtusifolius L.	13	21	11	1
Chenopodium album L.**	13	21	8	<1
Cirsium arvense (L.) Scop.**	7	10	9	1
Atriplex patula L.**	7	13	3	<1
Petunia hybrida Vilm.**	14	19	76	4
Nicotiana clevelandii Gray**	16	23	37	2
Chenopodium quinoa Willd.**	11	14	1	<1
C. amaranticolor Coste & Reyn.**	14	20	12	1
Humulus lupulus L.**	10	13	1	<1
No host	13	13	0	0

*Conducted in 90 ml clay pots from May-July, 1970; inoculum was 20 males and 30 females per plant; mean of 8 replicates.

†L1, L2 and a negligible number of uterine eggs.

**Host of AMV-H (see Table 15).

cylindrical tubes (Pitcher and McNamara, 1970) containing 35 ml soil in which less than 50% of the nematodes survived with hop, after 10 weeks, and together with Chenopodium quinoa and C. amaranticolor showed low reproductive potential compared with Petunia hybrida and Nicotiana clevelandii. Stellaria media, probably the most common of the weeds evaluated, gave the highest reproduction for the nematode, with six larvae per female, followed by P. hybrida, the best among the indicators, with four. Nematode reproduction was poor on Anthemis cotula, although it gave the highest inoculum survival, followed by N. clevelandii and Poa annua. All species of Chenopodium tested, and also Atriplex patula, were poor hosts in supporting nematode reproduction.

All the test plants produced roots with galls and distortions in varying degrees, shapes and sizes as a result of the nematode feeding. The relative sizes of the galls formed by each species are shown in Figs. 7-9. Those that formed galls readily and abundantly were S. media, P. annua, P. hybrida and Senecio vulgaris. Hop, N. clevelandii, Rumex obtusifolius, Cirsium arvense, Atriplex patula and all the Chenopodium species did not form many galls, even with high nematode inoculum. Galls formed by S. media and S. vulgaris were large and visible to the naked eye; a lens was needed to detect galls in the other species. Urtica urens, also a common weed in hop fields, formed few, but relatively large galls (Fig. 8D).

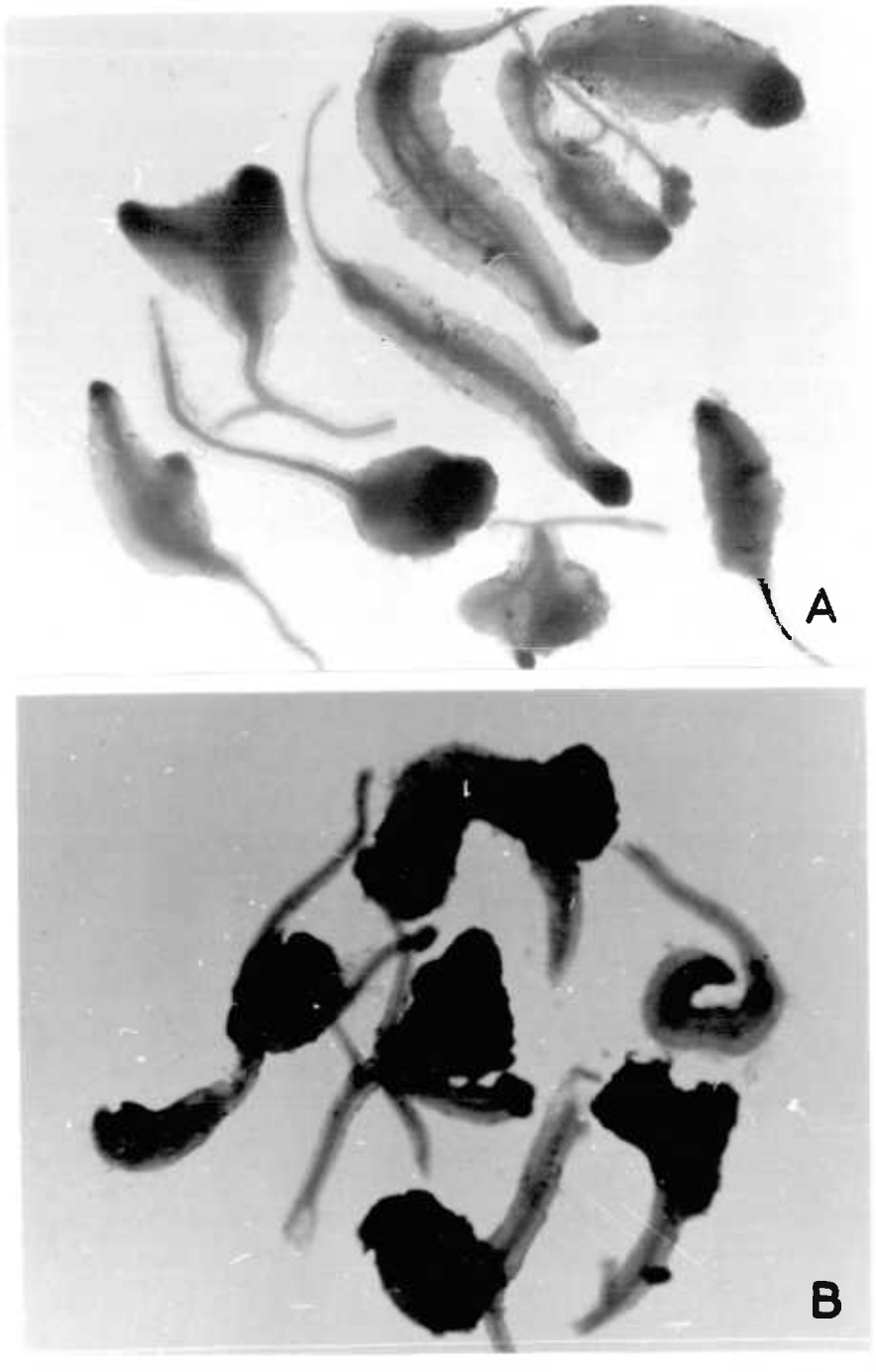


Fig. 7. Root galls induced by Xiphinema diversicaudatum on (A) Stellaria media, (B) Senecio vulgaris.

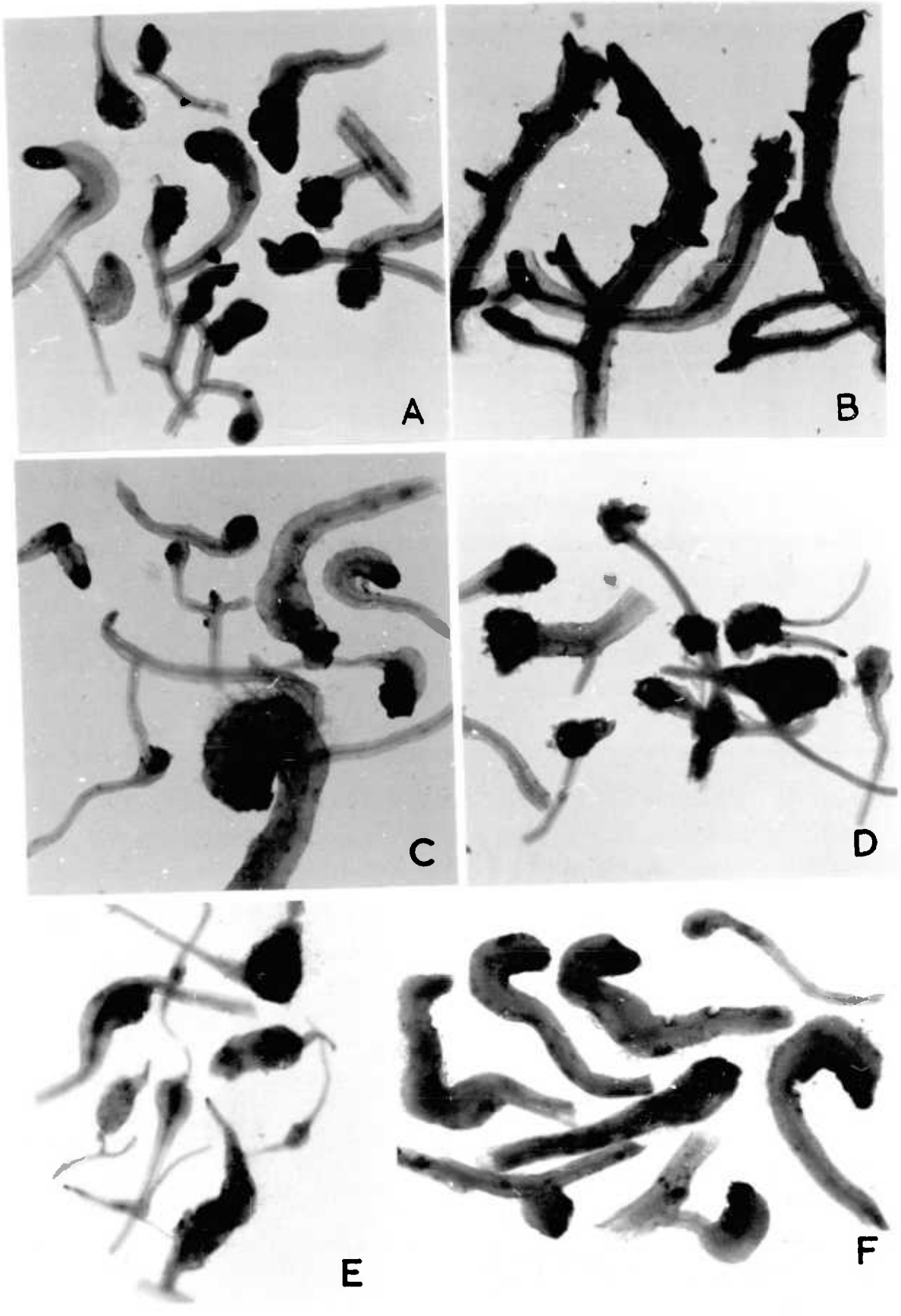


Fig. 8. Root galls induced by Xiphinema diversicaudatum on (A) Petunia hybrida, (B) Humulus lupulus, (C) Sonchus oleraceus, (D) Urtica urens, (E) Atriplex patula, (F) Anthemis cotula.

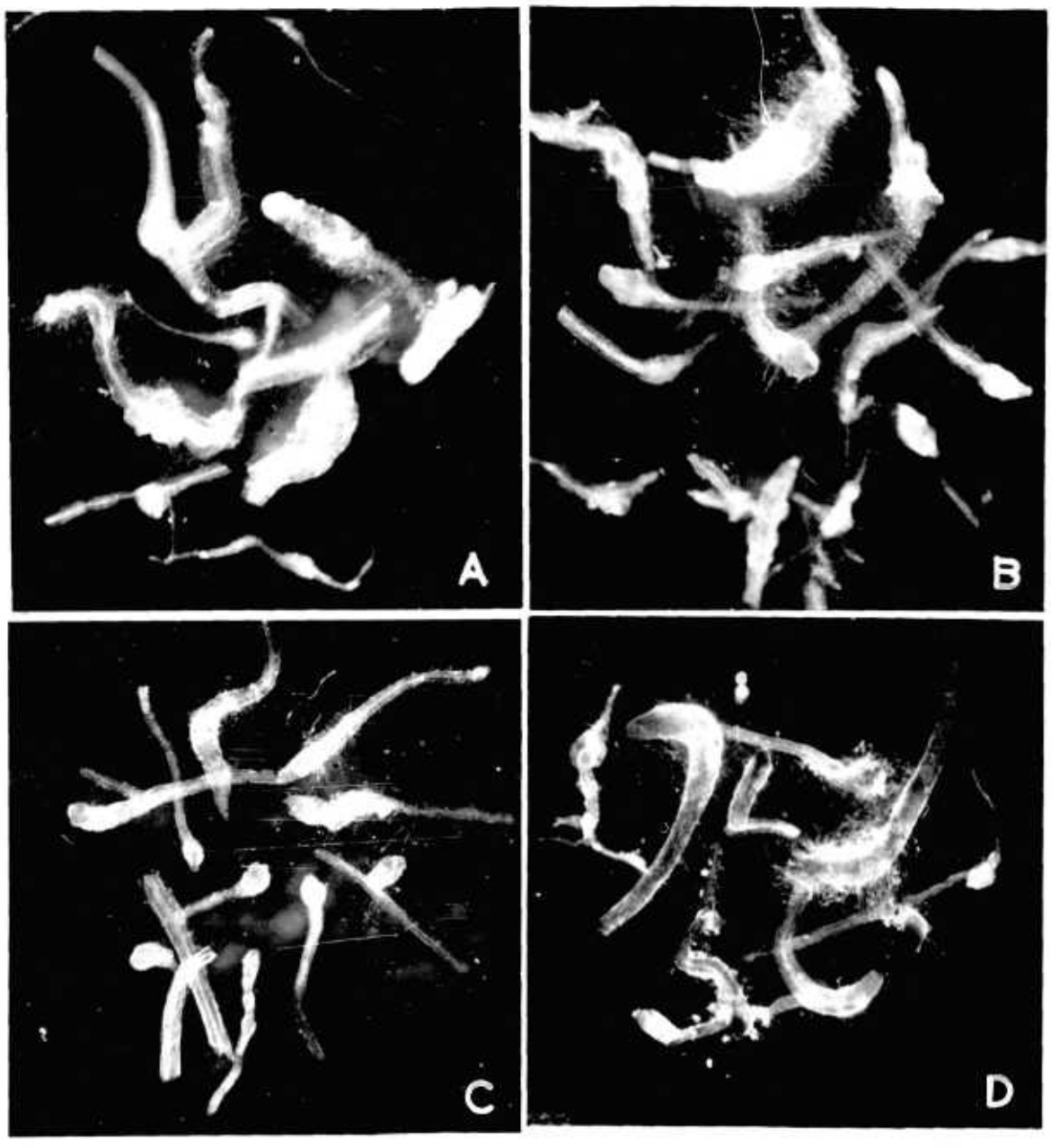


Fig. 9. Root galls induced by Xiphinema diversicaudatum on (A) Chenopodium quinoa, (B) C. amaranticolor, (C) C. album, (D) Pea annua.

Evidence of feeding on hop roots.- Feeding of nematodes on plant roots may provide circumstantial evidence of their role in the etiology of a virus disease. Thus, gall formation induced by known vectors or multiplication of such nematodes in the crop plant serve as clues to their virus-vector potential. However, some species, e.g. hop, neither form galls nor support much nematode multiplication. This study, aimed at detecting X. diversicaudatum feeding on hop roots, was conducted in the early part of 1969, when the relationship of X. diversicaudatum and AMV-H was not yet established.

Rooted shoot-tip cuttings of hops grown in polythene ice-cube moulds, containing sterilized soil, were inoculated with hand-picked X. diversicaudatum. After 4 weeks, the root system of each plant was washed gently into a beaker of water and then examined immediately in a Petri dish under a stereoscopic microscope. A number of adults and fourth stage larvae were observed with their stylets embedded in the root tissue. In one case, four nematodes were found on one plant. A short portion of the root containing each nematode was cut out, the nematode was relaxed with heat and, together with the root, stained in cotton blue-lactophenol (Goodey, 1963). The results are shown in Figs. 10-11. The nematodes feed on any portion of the root, with the lip region partly embedded and the stylet, which often penetrated intracellularly, reaching the vascular region in smaller roots, was, in most cases, curved in the tissue. Bending of the stylet in root tissue has been reported on petunia by Pitcher and Posnette (1963). In hop, slight discolouration of cells around the stylet was observed in a few instances.

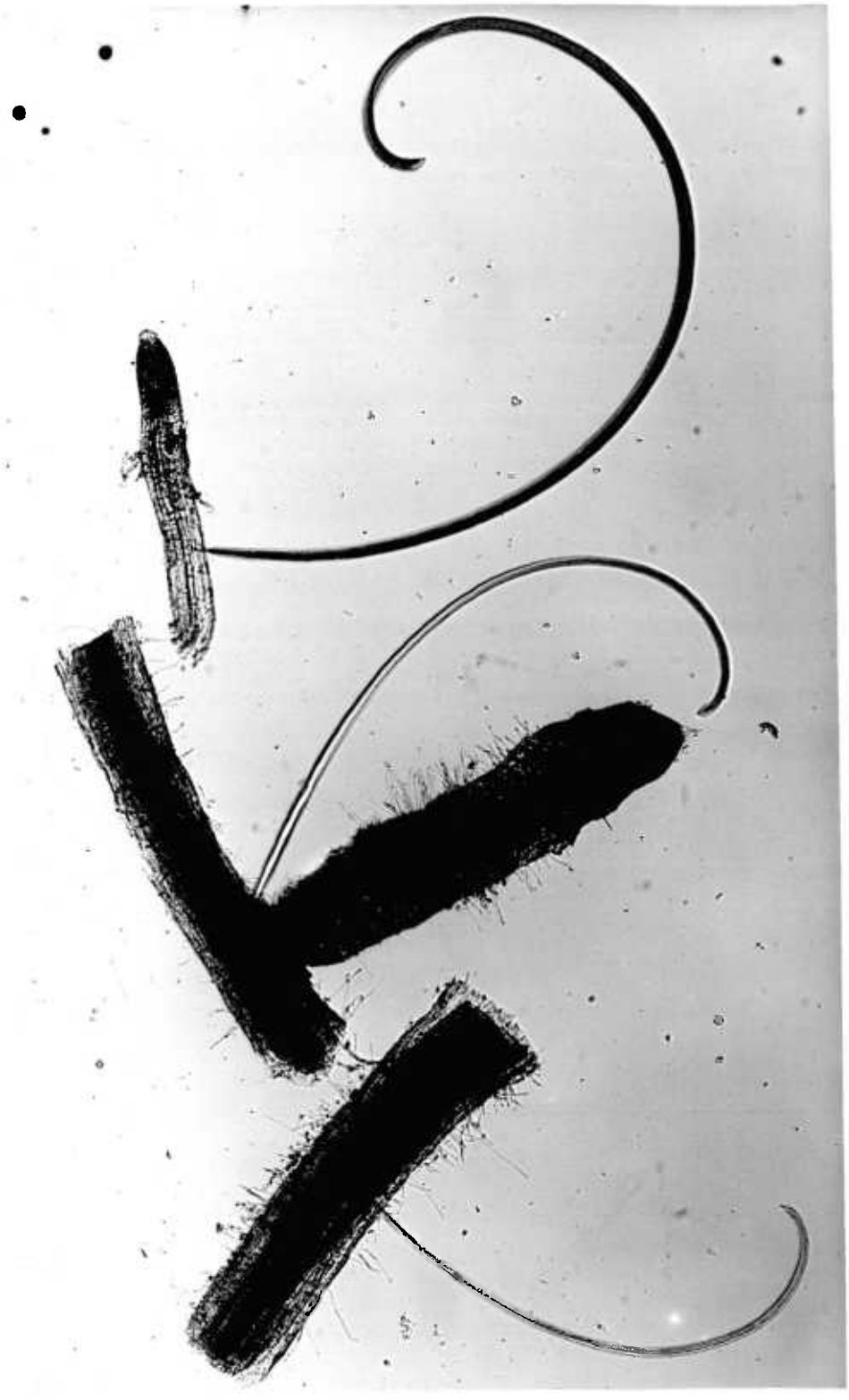


Fig. 10. Portions of hop roots with adult and 4th stage larvae of Xiphinema diversicaudatum trapped in the act of feeding.

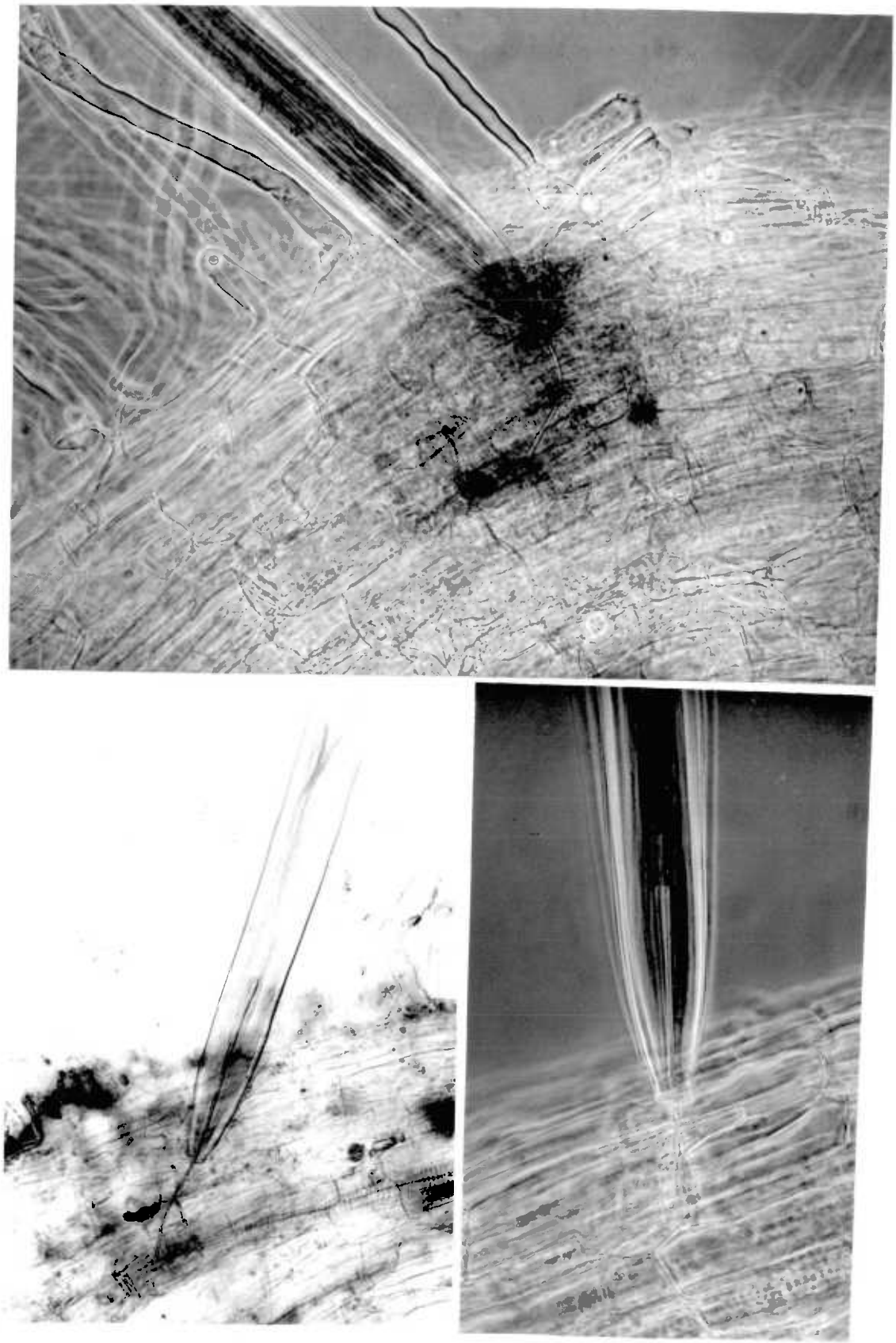


Fig. 11. Xiphinema diversicaudatum feeding on hop roots with lips partly embedded and with stylets extruded in varying positions; note replacement stylet (below, left) of 4th stage larva.

VIRUS-VECTOR RELATIONSHIPS OF AMV-H AND XIPHINEMA DIVERSICAUDATUM

Virus detection

Symptomatology of test plants

The symptoms of AMV-H on C. amaranticolor and C. quinoa were first described by Bock (1966). Additional symptoms have been observed on these two species as well as on C. murale and C. album, all of which were used extensively in this study. The symptoms described occurred on test plants that were sap-inoculated with either AMV-H (S) or AMV-H (N) isolates and were incubated at 14-18°C in the growth chamber. Both isolates showed similar symptoms in each test plant species under these conditions, but symptoms were rare in fluctuating glasshouse environment.

C. amaranticolor.- Vigorous plants sap-inoculated at 6-leaf stage showed systemic salt-mosaic symptoms usually after 9-10 days starting at the base of young leaves, accompanied by slight downward cupping and vein-banding (Fig. 12A). This salt-mosaic pattern disappeared in about 2 days and vein-net symptoms followed, together with chlorosis, crinkling and wavy leaf margins (Fig. 12B). Only 3-4 leaves usually show systemic symptoms. After 3-4 weeks, these leaves eventually became green, but could still be distinguished by the characteristic crinkling and wavy margins. By contrast, the strawberry isolate of AMV-T caused small necrotic lesions on inoculated leaves and death of the shoot after one week (Fig. 13A).

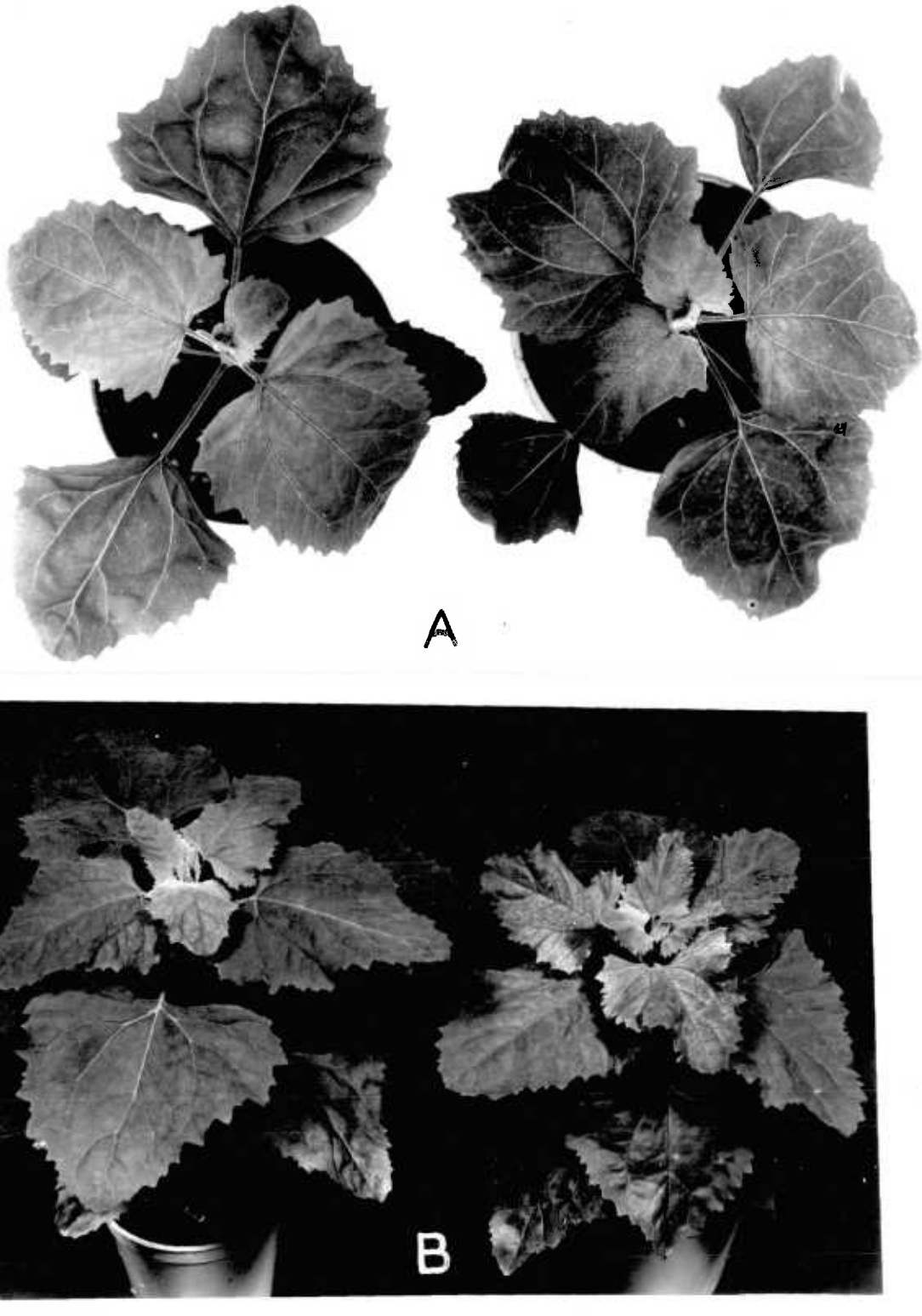


Fig. 12. Symptoms caused by AMV-H on Chenopodium amaranticolor at 14-18°C.
A, right: slight downward cupping accompanied by salt-mosaic and vein-banding of young leaves, 12 days after sap-inoculation.
B, right: characteristic chlorosis of young leaves with wavy margins, 3 weeks after inoculation.
Contrast flat leaves of healthy controls at left.

Test plants inoculated at 10-12-leaf stage show only the vein-net symptoms accompanied by mild chlorosis. Bending of the shoot and mild chlorosis of young leaves sometimes occur on a small proportion of plants.

C. album.- Only the systemic salt-mosaic symptoms and the slight downward cupping of young leaves described in C. amaranticolor occur. The symptoms disappear within a few days.

C. quinoa.- Bock (1966) described symptoms on C. quinoa as 'mild vein-net progressing to brilliant yellowing, light leaf distortion and rarely by raised dark green blisters'. In repeated sap-inoculation tests, where the plants were incubated in the greenhouse and in the growth chamber to determine which was the better indicator between C. amaranticolor and C. quinoa, the yellowing symptoms reported by Bock were reproduced in only one instance - a batch inoculated with the AMV-H (S) isolate, although the virus was also detected in symptomless plants either by infectivity or by gel-diffusion serology tests. Parallel tests on C. amaranticolor consistently gave distinct symptoms in all tests. However, a more characteristic and reproducible symptom of AMV-H on C. quinoa is the downward bending of the lower leaves to bring their tips close to the stem (Fig. 13B).

C. murale.- Both the AMV-H (S) isolate and the AMV-H (N) isolate, when sap-inoculated separately to vigorous C. murale at 6-leaf stage, cause progressive infection resulting in eventual death of all shoots. The first systemic symptoms appear 2-3 weeks from inoculation as yellowing and distortion

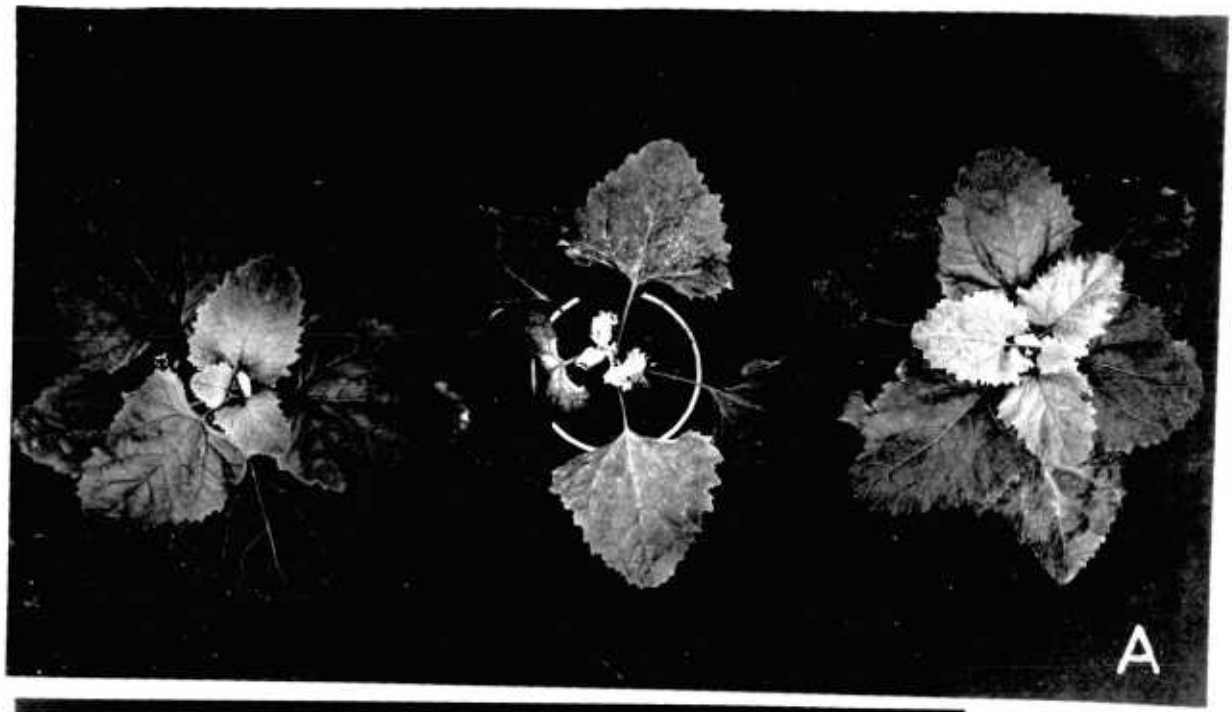




Fig. 13A. Comparison of symptoms of AMV-H after 3 weeks (right) with those of AMV-T after 2 weeks (centre) which causes minute necrotic lesions on sap-inoculated leaves and death of shoot. Left: healthy.

Fig. 13B. Right: Symptoms of AMV-H on Chenopodium quinoa at 14°C showing yellowing of top leaves and bending of lower leaves those to the stem. Left: healthy.

of the youngest leaves (Fig. 14A). Subsequently, the primary shoot dries up, followed by the lateral shoots, progressing downwards, and finally ending in 'blind tips', leaving the older leaves green. Unlike the infection caused by the type strain of AMV, where the plant collapses and dies in 2 weeks, a plant with 'blind tips' can live for a long time although it is unable to produce new shoots. Plants inoculated when slightly older usually produce seeds, although, in time, subsequent shoots also die. In a batch of 6 plants each inoculated in February, 1970, with both AMV-H (S) and AMV-H (N) isolates and incubated in the greenhouse, a progressive bright yellowing symptom occurred on the older leaves 5 weeks from sap-inoculation, after all the tips had died. The symptoms started at the base of the leaf and gradually moved towards the tip, preceded by yellow lesions (Fig. 14B).

Detection of AMV-H in C. amaranticolor

Detection of infected plants in nematode transmission tests was beset by problems at the start of the work due to the mild symptoms induced by AMV-H. Growth of test plants during winter, when temperatures in the greenhouse are favourable for the virus, was slow and poor due to insufficient lighting. The mild symptoms produced on herbaceous indicator plants were masked by high temperatures in the greenhouse that sometimes occur as early as late March. Similarly, detection by gel-diffusion serology usually failed unless done on plants showing distinct systemic symptoms. The following experiments report studies on the

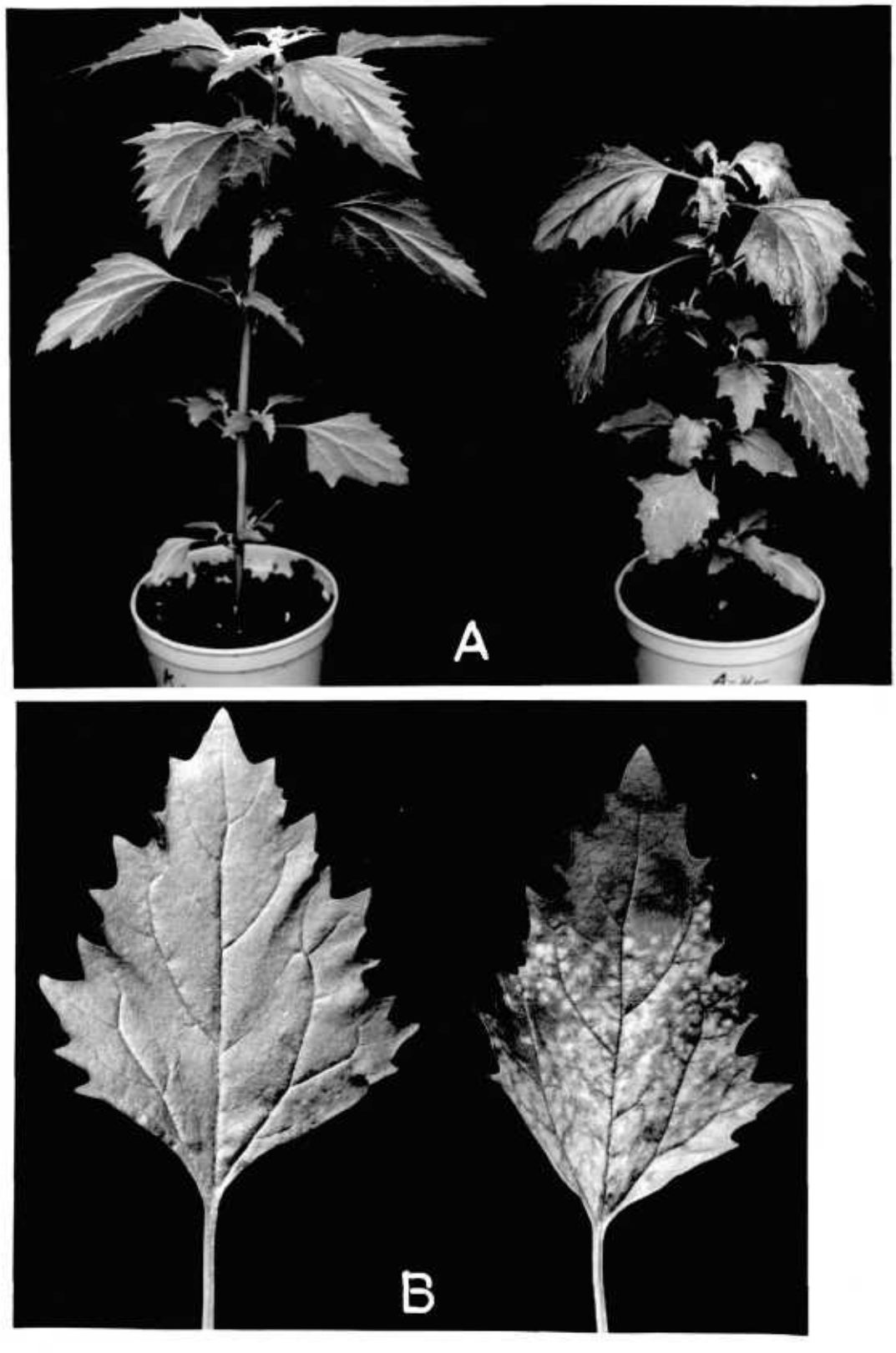


Fig. 14. Symptoms caused by AMV-H on Chenopodium murale showing (A) wilting of tips, 3 weeks after inoculation and (B) close-up of yellowing on older leaves developing 5 weeks after inoculation; healthy controls on left.

relationships between symptom expression and (a) size of test plant, (b) environmental temperature, and (c) timing of gel-diffusion serology on test plants infected with AMV-H.

(a) Size of test plants.- The plants tested were 7 cm and 15 cm tall, measured from soil level to shoot tip, being 5 and 7 weeks old from sowing and with 6 and 12 true leaves, respectively. All leaves were inoculated with the same inoculum, obtained from roots and shoots of infected C. amaranticolor, ground with Celite. The plants were incubated in the growth chamber at 14-18°C and gel-diffusion serology was done on 6 or 12 plants of each treatment 18 days from sap-inoculation.

Systemic salt-mosaic symptoms developed on young leaves of the smaller test plants 9 days from sap-inoculation, followed by vein-net and slight downward cupping of young leaves and later by chlorosis and crinkling of the leaf margins of 3-4 subsequent leaves that remained distinct until the eighteenth day. Symptoms on the bigger test plants appeared 14-16 days from sap-inoculation and were characterized by mild vein-net and, later, chlorosis starting at the base of young leaves. A few plants showed a characteristic bending of the systemically infected shoot.

There was no difference between the density of precipitation lines formed by sap of the smaller and bigger test plants in gel-diffusion serology tests. However, the leaves of the bigger plants were harder to grind and yielded much less sap.

(b) Environmental temperature.- There are many reports, e.g. Holmes (1964), that the symptoms of many viral diseases are masked at high environmental temperatures. A practical solution offered by Ross (1964) was to conduct the experiments when near-normal temperatures can be maintained and when conditions are not limiting for plant growth. The masking effect of temperature on nettlehead was reported by Keyworth (1941) on hops grown in warm greenhouses and on plants out-of-doors during hot summers. Hence, Keyworth and Paine (1950) recommended that roguing for nettlehead be done very early in the season before the bines are trained.

The effects of winter and summer temperatures in the greenhouse compared with a controlled temperature in the growth chamber on symptom expression of AMV-H in C. amaranticolor are shown in Table 9. The test plants were at 6-true-leaf stage and were inoculated with similar inoculum ground with Celite from roots and shoots of infected C. amaranticolor. No symptoms developed on plants inoculated in June and July incubated at 13-28°C in the greenhouse, whereas their counterparts incubated at 17-19°C in the growth chamber developed distinct symptoms in 12-15 days and gave positive reactions in gel-diffusion serology. In plants inoculated in July that gave no symptoms and serological reactions, two out of 10 proved to be infected when the roots were later inoculated to C. amaranticolor plants which were incubated in the growth chamber. Test plants incubated in a Fisons growth cabinet at 15°C and 80% R.H. developed salt-mosaic symptoms in 10 days similar to those in the growth chamber. However, the symptoms were soon masked, apparently

Table 9. Effect of temperature on symptom expression of AMV-H in Chenopodium amaranticolor

Inoculated	Greenhouse			Growth chamber		
	Temp. (°C)	Symptoms appeared (days)	Rate with symptoms*	Temp. (°C)	Symptoms appeared (days)	Rate with symptoms*
3.VI.70	19-28	No symptoms	0/15	17-18	12	15/15
13.VII.70	13-28	No symptoms	0/12	17-19	13	12/12
8.X.70	-	-	-	15†	10	20/20
8.X.70	-	-	-	14-19	10	20/20
1.II.70	16-18	13	12/12	14-16	13	12/12
4.II.71	16-18	13	15/15	14-16	10	15/15

*Numerator is the number of plants that developed symptoms and the denominator is the total number of plants sap-inoculated.

†Conducted in Fisons (Type 1200 FG) growth cabinet.

due to the 2,000 f.c. light intensity that caused stunting of the plants which developed thick, leathery and deep-purple leaves. Nevertheless, such plants gave good serological reactions. All the plants inoculated in February and incubated in greenhouse or growth chamber developed very distinct symptoms in 10-14 days.

(c) Timing of gel-diffusion serology.- The test plants used in the experiments given in Table 10 had symptoms of similar severity. They were all incubated in the growth chamber kept at 13-18°C between sap-inoculation and serology test. In all cases, only the young leaves were tested, including the healthy controls of each experiment. The first test, 18 days after inoculation, gave 100% positive reaction. In other routine tests, similar results have been obtained after 16 days with more vigorous test plants or after 20 days, as shown in Experiment IV in Table 10. In Experiment I, when the average temperature in the growth chamber was 18°C, the test plants grew faster, subsequent leaves became symptomless and the number of plants giving a positive serological reaction was reduced. However, when the average temperature was 13°C, as in Experiment II, growth rate was decreased, symptom expression was prolonged and 50% of the plants still gave positive serological reaction after 28 days. The virus was not detected 35 days after inoculation.

Table 10. Timing of gel-diffusion serology for detection of AMV-H in Chenopodium amaranticolor

Expt. No.	Interval between inoculation and serology (days)	Positive reaction / Plants tested
I	18	6/6
	22	2/6
	28	1/6
II	18	12/12
	22	10/12
	28	6/12
	35	0/12
III	28	1/14
IV	20	15/15
V	18	20/20

Transmission of AMV-H from hop to Chenopodium quinoa and to hop.- The results of three experiments conducted to determine whether X. diversicaudatum transmitted AMV-H, using C. quinoa and hop as bait plants are given in Table 11. Rooted shoot-tip cuttings of healthy hop var. Bramling M (meristem clone) were used in Experiment I after Dr. J.M. Thresh (personal communication) found AMV-H, for the first time, by serology, in some of the Fuggle hop seedlings being used in transmission experiments. In Experiment I (Table 11) when non-infective nematodes were hand-picked, given 8 weeks of acquisition access feed on rooted shoot-tip cuttings from nettlehead-diseased hops grown in polythene ice-cube moulds and then transferred to rooted shoot-tip cuttings of healthy Bramling M at 60 per plant in polythene ice-cube moulds, they infected 40% and 21% of the C. quinoa and hop plants, respectively. In Experiment 2, where 'field nettlehead Xiphinema' were transferred at 30 per plant using plastic egg cups, there was 50% and 10% transmission to C. quinoa and Fuggle hop seedlings, respectively. In Experiment 3, the figures for each test plant and for each stage of nematode inoculum represent the combined totals of two trials. The nematodes used, 'Slade box Xiphinema', were given at least 17 weeks acquisition access feed. Those used in the first trial of each test plant were contained in plastic egg cups; those in the second trial were transferred to small clay pots. The results showed that both adults and larvae, kept separately at 50 per plant in either egg cups or small clay pots, transmitted AMV-H equally. Harrison and Winslow (1961)

Table 11. Transmission of AMV-H by *Xiphinema diversicaudatum* from hop to *Chenopodium quinoa* and to hop

Expt. No.	Initial status of nematodes	Acquisition access (wks)	Nematode		% Transmission	
			Per Plant	Stage	*C. quinoa*	Hop
1	Non-infective	8	60	AD,L3,L4	40 (6/15)	21 (3/14)
2	Infective	Since L1	30	AD,L3,L4	50 (10/20)	10 (2/20)
3	Non-infective	17+	50	AD	100 (30/30)	100 (20/20)
3	Non-infective	17+	50	L3,L4	100 (20/20)	100 (17/17)
Control	Non-infective	0	100	AD	0 (0/20)	0 (0/10)
	-	-	0	-	0 (0/40)	0 (0/30)

*Figures in parentheses denote the number of plants infected/total number inoculated.

reported a higher percentage of transmission of AMV by adult X. diversicaudatum than by larvae. Similarly, Taylor and Thomas (1968), obtained transmissions with females, males and juveniles.

Results of gel-diffusion serology tests made on all the C. amaranticolor indicator plants showing systemic symptoms in Experiments I, II and III confirmed the presence of AMV-H. None of the control plants were infected. The results of these three experiments, therefore, proved that X. diversicaudatum is a vector of AMV-H.

Transmission of nettlehead disease to hop.- Table 12 shows the results of transmission trials of nettlehead and line-pattern diseases by X. diversicaudatum. The nematodes used, Nettlehead soil box Xiphinema, were sieved out after an acquisition access feed of about a year, hand-picked and transferred to hop seedlings in egg cups at various rates (Table 13) in April, 1970. The bines were cut back in September, 20 weeks after inoculation, and the plants placed in a cold store for 14 weeks at 3°C ('winter treatment'). Symptoms of nettlehead and line-pattern developed between February 10 and March 6, 1971, i.e. 4-7 weeks after the plants were returned to the greenhouse at 17°C. Nine out of 40 plants (22.5%) developed nettlehead symptoms and one out of 40 showed line-pattern symptoms (Fig. 15). In another experiment, where virus-free X. diversicaudatum were given 3 months acquisition access feed on rooted shoot-tip cuttings of nettlehead-affected hops, sieved out, hand-picked and transferred to healthy Fuggle hop seedlings grown in egg cups, at the rate of 50 adults and larvae per plant, one out of 8 plants showed nettlehead symptoms after 7 months.

Table 12. Transmission of nettlehead and line-pattern diseases to hops by *Xiphinema diversicaudatum**

Symptoms	No. plants showing symptoms	No. plants infected with AMV-H
Nettlehead	9	9
Line-pattern	1	1
None	30	22
Total	40	32

*Nematodes raised on nettlehead-diseased hops.

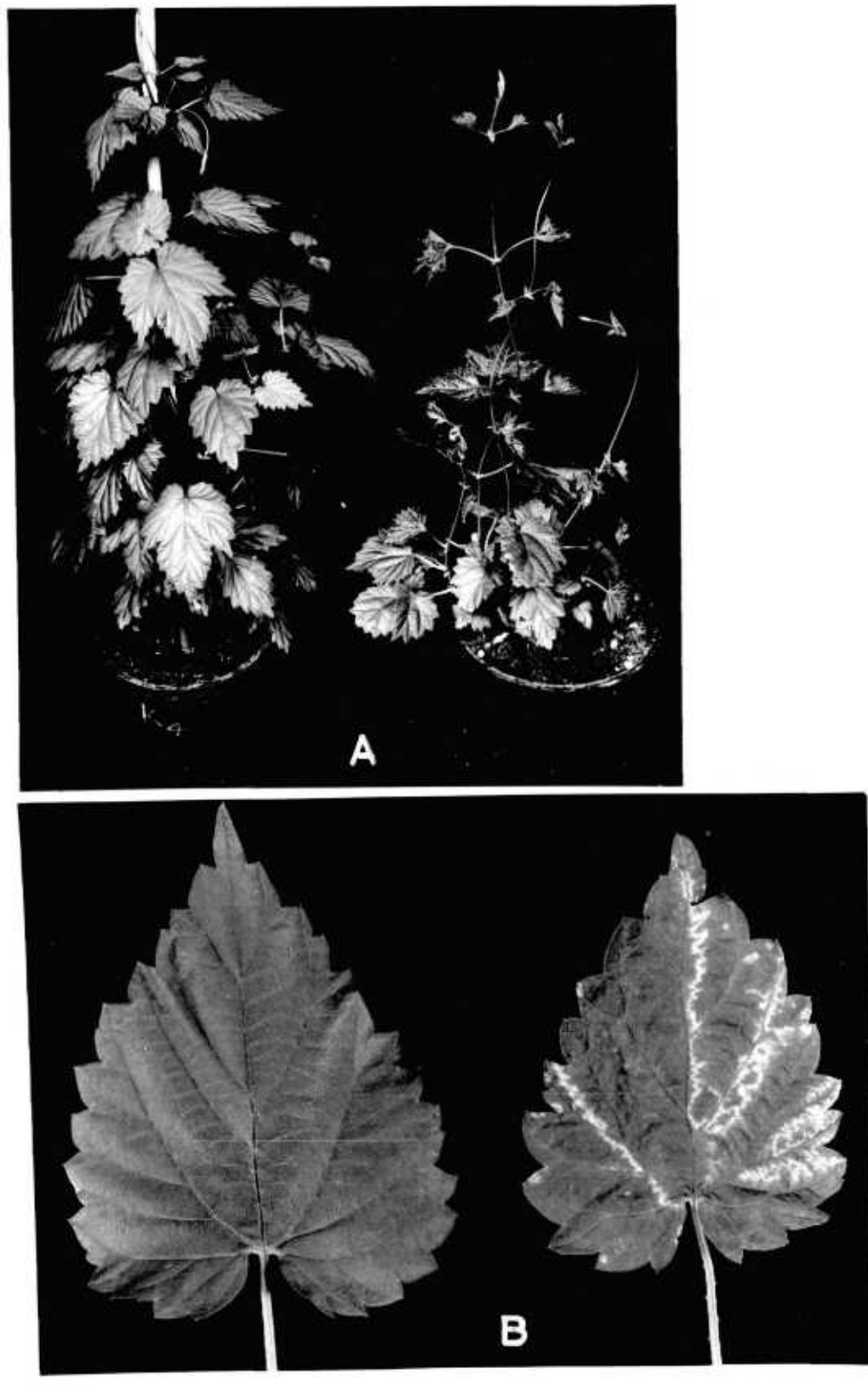


Fig. 15. Fuggle hops (F.37 M). (A) Right: Nettlehead transmitted by hand-picked Xiphinema diversicaudatum raised on nettlehead-affected hops. Left: Healthy plant, infested with non-infective nematodes.

B) Right: Leaf symptoms of hop line-pattern in seedling of Fuggle (F.37 M) transmitted by hand-picked X. diversicaudatum. Left: healthy.

All the test plants were assayed for AMV-H, first, by an infectivity test, where roots were ground with Celite and inoculated to C. amaranticolor 20 weeks after nematode inoculation and, second, by gel-diffusion serology, carried out in January, 1971 on new growth following the winter treatment. In both tests, AMV-H was present in the plants showing symptoms of nettlehead, but it was not detected in the plant with line-pattern symptoms, or in another serology test done 2 weeks later. However, its presence was confirmed in two subsequent infectivity tests by rubbing young leaves showing line-pattern on to C. amaranticolor plants which became infected with AMV-H (confirmed serologically), indicating that infectivity test is more sensitive than gel-diffusion serology. Of the 30 plants that remained symptomless, 22 were infected with AMV-H and 8 plants were not infected.

Necrotic ringspot virus (NRSV) was absent from all the test plants that showed nettlehead or line-pattern symptoms when assayed on cucumber, indicating that the nematodes did not transmit this virus, which was present in the infector plants. Also, NRSV was not implicated in nettlehead as line-pattern disease.

Effect of number of X. diversicaudatum on the transmission of AMV-H and nettlehead disease.- Jha and Posnette (1961) showed that one adult X. diversicaudatum could transmit the type strain of arabis mosaic virus (AMV-T) and that approximately one-third of the nematode population, fed on strawberry infected with arabis mosaic, infected petunia test plants. Table 13 shows the effect of number of X. diversicaudatum on

the transmission of AMV-H and nettlehead using C. amaranticolor, C. quinoa and hop as test plants. The 'Slade box Xiphinema' used for these tests were sieved out, hand-picked in lots of 1, 5, 10, 25, 50 and 100 and transferred to the test plants in egg cups containing 2:1 steamed soil-sand mixture. The nematodes used at rates 1, 5 and 10 per plant, including those in the non-infective controls, were adults only; those used at the other rates consisted of 50% adults and 50% larvae (L3, L4). After 3 weeks of transmission access feed, the test plants were transferred to pots of steamed soil to allow further root growth and virus multiplication, for 5 weeks each for C. amaranticolor or C. quinoa, and 17 weeks for hop, before the roots were assayed on C. amaranticolor indicators. The virus in systemically-infected indicators was confirmed by gel-diffusion serology.

Two out of 8 of the C. quinoa test plants inoculated with one adult nematode each were infected with AMV-H. No transmission occurred on either C. amaranticolor or hop inoculated with one nematode. At 5 nematodes per plant, transmission of AMV-H was obtained in all three species of test plants, with 12.5% infection of C. amaranticolor, 50% of C. quinoa and 62.5% of hop; transfers at the same rate gave 12.5% transmission of nettlehead. The low transmission rate of AMV-H to C. amaranticolor was possibly due to poor growth of the test plants resulting from inadequate lighting in the greenhouse during the test in December to January rather than to inherent resistance. Transmission of either AMV-H or nettlehead at 25 nematodes per plant was about as

Table 13. Effect of number of Xiphinema diversicaudatum on the transmission of AMV-H and nettlehead

Nematodes per plant	Test plant infected out of 8			
	Chenopodium amaranticolor	Chenopodium quinoa	Humulus lupulus	
			AMV-H	Nettlehead
1	0	2	0	0
5	1	4	5	1
10	2	Not done	6	1
25	3	7	8	3
50	2	8	7	3
100	2	8	7	1
0	0	0	0	0
100 Non-infective	0	0	0	0

high as at either 50 or 100 nematodes per plant. All the control plants remained healthy in the three experiments.

Interval between nematode inoculation of virus and detection in youngest leaves.- An important feature of the spread of nettlehead in the field, pointed out by Keyworth and Paine (1950), was that hop plants do not usually show symptoms until the year after infection. In order to counteract the effects of this time-lag factor on roguing, Keyworth and Davies (1946) recommended removal of the neighbours of diseased plants, as well as those obviously infected.

To determine how soon AMV-H, transmitted by X. diversicaudatum to young hop seedlings and three species of Chenopodiaceae, would move to and be detectable in young leaves, infectivity tests to C. amaranticolor or serology tests were made at intervals (Table 14). Growth was stimulated by repotting the test plants in steamed compost and periodic pruning. In the first test on C. quinoa, AMV-H was not detected in either serology or leaf infectivity tests made at 8, 11, 15 and 19 weeks after nematode inoculation. In the second test, however, infection was detected 15 weeks after nematode-inoculation, presumably due to the colder temperature in December, in contrast to the summer temperatures during the first test. The virus was detected in the leaves of C. album and Atriplex patula by infectivity tests done 16 weeks after nematode inoculation. In Test 1 on hop, AMV-H was not detected in gel-diffusion serology carried out 21 weeks after nematode inoculation; positive reaction was obtained in all test plants after 40 weeks when serology was conducted on new growths after chilling in a cold room. In

Table 14. Interval between inoculation with *Xiphinema diversicaudatum* and detection of AMV-H in youngest leaves

Host species	Nematodes inoculated (1970)	Reaction and test interval (wks.)		Infection rate
		-	+	
Chenopodium quinoa				
Test 1	Jan. 1	19	22	8/10
Test 2	Sept. 8	10	15	8/15
Chenopodium album	Aug. 25	9	16	5/6
Atriplex patula	Aug. 25	9	16	4/6
Humulus lupulus				
Test 1	April 21*	21	40	20/20
Test 2	Sept. 21†	22	27	5/6

*Chilled in cold store for 14 weeks.

†Vegetative growth maintained in heated greenhouse.

Test 2, however, where growth of the hops was maintained throughout the winter under artificial light in the greenhouse, the virus was detected serologically in the youngest leaves 27 weeks after nematode inoculation, indicating that a period of dormancy or chilling is not essential for virus movement from the roots to the aerial parts. These results agree with the findings of Fulton (1941) who showed that the upward movement of virus from inoculated roots was very slow and restricted in contrast to the relatively rapid rate of downward movement from inoculated leaves.

Transmission of AMV-H to weeds and to virus indicators.- The ability of vector nematodes to survive long periods without food and their polyphagous nature not only ensure their survival in soils but also the perpetuation and spread of the viruses they transmit (Harrison, 1964; Murant, 1970). Legg (1964) sap-inoculated 28 herbaceous species of virus indicators and weeds with extracts of nettlehead-diseased hops, but failed to detect any virus specific to nettlehead. Using some of the indicator plants tested by Legg, Bock (1966) reported C. amaranticolor and C. quinoa as apparently the only hosts of AMV-H, which he isolated and identified from both nettlehead-diseased and some symptomless hops. In my attempts to find better indicators for use in nematode transmission experiments and to establish the role of weeds on the ecology of AMV-H, I evaluated some of the plants tested by Legg (1964) and by Bock (1966) along with weeds considered by A.D.A.S. hop specialists, N. Moss and C.D. Reekie, to be common in hop fields. This was done both by manual

inoculation and with X. diversicaudatum, using the AMV-H (S) isolate. Virus-free seeds of weeds belonging to seven families, viz., Caryophyllaceae, Chenopodiaceae, Compositae, Gramineae, Onagraceae, Polygonaceae and Urticaceae, collected at East Malling Research Station were used. All species, except those with narrow leaves, such as Anthemis cotula, Matricaria matricarioides, Poa annua and Stellaria media, were inoculated with sap from shoots or roots of infected C. amaranticolor. Inoculation by hand-picked X. diversicaudatum was done at the seedling stage in egg cups, containing 2:1 soil-sand mixture, at 60 adults and larvae (L3, L4) per plant. At least three inoculation trials were made on the test species that gave negative transmissions (Table 15).

Table 15 shows eight new hosts of AMV-H, viz., Chenopodium album, C. murale, Nicotiana clevelandii, Atriplex patula, Senecio vulgaris, Cirsium arvense, Fragaria vesca and Petunia hybrida; all were infected by means of X. diversicaudatum and six were also infected by sap-inoculation. Among the weeds, C. album, A. patula and S. vulgaris were equally susceptible to AMV-H. No infection was obtained in repeated sap-inoculations of P. hybrida, although it was easily infected by infective nematodes. Virus transmission by nematodes occurred in another test in which petunia were grown for 6 weeks in a box containing infective X. diversicaudatum. Thomas (1970) reported S. media, P. annua and M. matricarioides as hosts of the type strain of arabis mosaic virus, but repeated tests using AMV-H were unsuccessful, thus providing additional evidence of strain differences between AMV-H and AMV-T. The roots of Rumex

Table 15. Transmission of AMV-H to some virus indicators and to weeds common in hop fields

Test plants	Number infected by	
	Sap-inoculation, out of 8	Xiphinema, out of 12*
Chenopodium quinoa Willd.	8	12
C. amaranticolor Coste & Reyn.	8	7
C. murale L.	8	10
Nicotiana clevelandii Gray	8	12
Petunia hybrida Vilm.	0	12
Fragaria vesca L.	-	6
Atriplex patula L.	8	10
Chenopodium album L.	8	8
Senecio vulgaris L.	2	11
Cirsium arvense (L.) Scop.	0	6
Anthemis cotula L.	)	
Epilobium adenocaulon Hausskn.	)	
Matricaria matricarioides (Less.) Porter	)	
Poa annua L.	)	
Rumex obtusifolius L.	) +	
Stellaria media (L.) Vill.	)	
Taraxacum officinale Weber	)	
Urtica urens L.	)	

*Feeding in egg cups at 60 adults and larvae per plant.

+No infection after three trials.

obtusifolius, when sap-inoculated to C. amaranticolor, caused severe phytotoxicity on the rubbed leaves, resulting in scorching and eventual defoliation; the addition of phosphate buffer or polyethylene glycol was of very little help in reducing the damage.

Transmission of AMV-H through seed.- Seed transmission of AMV-H was obtained in Chenopodium album, C. quinoa and Atriplex patula plants that were inoculated at the seedling stage either with infective X. diversicaudatum or with infective sap (Table 16). In C. album infected by nematodes, the percentage of seed transmission based on 30 seedlings per plant of 5 plants tested, ranged from 36.7% to 70% (mean 54.5%). In two plants infected by sap-inoculation, seed transmission averaged 66.7% and inoculated C. amaranticolor indicators showed more severe systemic symptoms, suggesting a higher concentration of virus in the seeds compared with the seeds of plants infected by nematodes. These results approximate to the 80% seed transmission obtained by Lister and Murant (1967) on C. album sap-inoculated with AMV-T. Seeds harvested from C. quinoa or A. patula plants infected by nematodes gave 13.3% and 30% infection, respectively.

In a test on volunteer Fuggle hop seedlings collected at first true-leaf stage from around hops showing bare bine symptoms at Bomford Farm, Worcestershire, on 7th May, 1969 and planted in pots of steamed soil, gel-diffusion serology gave a positive reaction on 7 out of 12 plants tested. These results, supported by the absence of X. diversicaudatum in soil samples obtained from this site, gave additional evidence that AMV-H is transmitted through the seed of hops.

Table 16. Transmission of AMV-H through seed of infected plants

Plant No.*	% infected seedlings (out of 30)
Chenopodium album	
1-X	53.3
2-X	53.3
4-X	60.0
8-X	70.0
12-X	36.7
1-S	76.7
6-S	56.7
Chenopodium quinoa	
M-X	13.3
Atriplex patula	
M-X	30.0

*Plants were infected by *Xiphinema diversicaudatum* (X) or by sap-inoculation (S) at the first true-leaf and at the 6-leaf stage, respectively.

Persistence of AMV-H in starved nematodes.- Studies on the persistence of AMV-T in starved X. diversicaudatum by Jha and Posnette (1961), Harrison and Winslow (1961) and Taylor and Thomas (1968) showed that the nematodes remained infective for 30, 24 and 112 days, respectively. The results of an experiment, conducted from April, 1970 to February, 1971 on the persistence of AMV-H in starved X. diversicaudatum, are shown in Table 17. The nematodes used in this study were 'Nettlehead soil box Xiphinema'. The soil was crumbled and mixed thoroughly by hand and all the hop roots were removed before storage in a polythene bag, to prevent drying, in an outside room exposed to summer and winter shade temperatures. At the end of each inoculation interval, the nematodes were sieved out, hand-picked and transferred to C. quinoa test plants either separately as adults (AD) or larvae (L3, L4) or as mixture of adults and larvae at 50 per plant, using the egg cup method. After 5 weeks of transmission access feed, the root system of each bait plant was washed free of soil and nematodes, ground with Celite and inoculated to C. amaranticolor indicators. The virus in plants showing systemic symptoms was confirmed by gel-diffusion serology.

Table 17 shows that AMV-H was retained by adult X. diversicaudatum, but not by larvae, for up to 36 weeks when kept without plants. No transmission was obtained with either adults or larvae in a subsequent test, after 44 weeks of starvation. The percentage of transmission declined with the length of starvation period, from 90% in the initial test to 13% after 36 weeks. The low percentage of

Table 17. Persistence of AMV-H in *Xiphinema diversicaudatum* in fallow soil

Starvation period (wks.)	Stage of nematode inoculum	*C. quinoa* test plants	
		Transmission rate	%
0*	AD,L3,L4	9/10	50.0
8	AD,L3,L4	8/10	80.0
25	AD,L3,L4	3/12	25.0
29	AD	7/16	43.8
	L3,L4	3/8	37.5
32	AD,L3,L4	3/8	37.5
36	AD	2/15	13.3
	L3,L4	0/15	0.0
44	AD	0/12	0.0
	L3,L4	0/12	0.0

*Initial test made on April 11, 1970.

transmission during the third test interval at 25 weeks was probably due to poor growth of the bait plants rather than to decreased infectivity of the nematodes. Transmission with larvae was obtained after 29 weeks of starvation, but the percentage was lower than with adults.

Attempted transmission of AMV-T to hops.- Attempts were made in two separate experiments, spaced at four-month intervals, to transmit AMV-T to hop by X. diversicaudatum. In both tests, the infective nematodes, which had had an acquisition access feed of 3 months on infected Petunia hybrida, were sieved, hand-picked and transferred at 100 adults and larvae (L_3, L_4) per Fuggle hop seedling at the cotyledon stage, using the egg cup and small clay pot methods in the first and second tests, respectively. After 6 weeks of transmission access feed, the root system of each hop test plant was washed free of soil and nematodes, ground with Celite, and inoculated to C. quinoa. No infection occurred in either experiment of 10 replicates.

The nematode inoculum in the first experiment was re-sieved after the 6-week transmission access feed, hand-picked and transferred to petunia seedlings in egg cups at 50 per plant. After 3 weeks of transmission access feed, the roots were washed free of soil and nematodes, ground with Celite and inoculated to C. quinoa. All 10 plants were infected and reacted in gel-diffusion serology tests, indicating that the nematodes were still infective with AMV-T.

DISCUSSION

Although there is considerable information about the ecology and virus-vector relationships of X. diversicaudatum and the type strain of arabis mosaic virus (AMV-T) (Jha and Posnette, 1961; Harrison and Winslow, 1961; Taylor and Thomas, 1968), virtually none is available about the relationships of this vector and the strain of AMV that is present in hops (AMV-H). The reason is apparently not due to the lack of economic importance of the problem, because the seriousness of nettlehead has been realized by hop farmers since 1894 (Keyworth and Paine, 1950), but due mainly to the difficulty of working with a very mild strain of AMV. Evidently, temperature is the most critical factor governing symptom expression of AMV-H in Chenopodium amaranticolor indicators and of nettlehead disease of hop. Thus, no symptoms of AMV-H developed in C. amaranticolor kept in the greenhouse when the temperature fluctuated from 13-28°C from April to November, but symptoms developed consistently when plants were grown in a controlled temperature chamber at 14-18°C. Similarly, hops infected by hand-picked X. diversicaudatum developed nettlehead or line-pattern symptoms by February or March when kept in a greenhouse at 18°C. However, when the temperature began to fluctuate from 17-28°C, in early April, the symptoms were masked and test plants showing severe nettlehead subsequently grew more vigorous, with large, normal leaves; a similar phenomenon was reported by Keyworth (1941) and has since been encountered by many other workers.

Hop is not a good host for X. diversicaudatum, as shown by low survival counts and a reproductive potential of less than one larva per female, compared to six on Stellaria media or on raspberry (Taylor and Thomas, 1968) and seven in strawberry (Flegg, Baxendale and Popham, 1970). However, X. diversicaudatum will feed on hop, as demonstrated by several specimens trapped whilst feeding, with their stylets, penetrating, in some cases, as far as the vascular region. Schindler (1957) was the first to establish the production of root galls as a result of feeding by X. diversicaudatum, on roses and peanut; later, galls were also observed on roots of plants infected with AMV-T by Harrison and Winslow (1961), Thomas (1970) and by Taylor and Thomas (1968). In the present study, hand-picked X. diversicaudatum caused root galls of various sizes and shapes in several weeds and virus indicators, but not in hops.

In studies on the vertical distribution of X. diversicaudatum in root observation boxes, made 6 months after planting the hops, there was an apparent increase in the number of L4s and adults with increasing depth, indicating a relationship with root concentration. The decrease in the number of X. diversicaudatum inside the root-free soil cylinders, accompanied by an increase in the number outside that were exposed to roots, which occurred in samples taken 6 months after planting the hops, but not after 3 months, indicates some degree of attraction by the hop roots as well as indicating the length of time that it took the nematodes to cover the 12 cm distance between

samples. These results approximate to the rate of spread of X. diversicaudatum in an uncultivated woodland soil, calculated by Harrison and Winslow (1961) as 1 foot per year. Evidently, the rate of movement is very slow and it is doubtful whether the nematodes that occur deeper than 40 cm could move upwards each year to feed on roots of more suitable but shallow-rooted annual weed hosts. More probably, the nematodes found at such depths survive on hop roots which, during sampling studies, were found as deep as 90 cm.

The distribution of X. diversicaudatum in a hop field with much nettlehead disease was patchy and erratic; often fewer were obtained from soil around plants showing an advanced stage of the disease. There were more nematodes in sites 30 cm from the hop plant than at 90 cm away, either between rows or between hills. The greatest density found was at a depth of 11-30 cm and although numbers decreased with increasing depth and distance, enough to cause virus infection was still present at 79 cm depth and at 90 cm from the hop plant. These findings are of importance in taking soil samples to assess the nematode population and when applying chemicals aimed at eliminating these nematode vectors.

In numerous tests with adults and larvae, X. diversicaudatum has been shown to transmit AMV-H to hop and to C. quinoa bait plants. Using hand-picked nematodes raised on nettlehead-diseased hops, it has been established for the first time that X. diversicaudatum can transmit the full syndrome of nettlehead. Thus, the early suspicions of hop growers that nettlehead persists in the soil

(Keyworth and Paine, 1950) have been proved right and the long search for its natural vector has finally succeeded. However, whether other viruses, such as one causing line-pattern, have been transmitted by the nematodes, besides AMV-H, must be determined by future research. Of the 40 plants inoculated in one test, 32 were infected with AMV-H. Of these, 9 developed nettlehead symptoms, only one showed line-pattern symptoms and 22 remained symptomless. It is possible that additional plants will develop nettlehead symptoms later as the summer glasshouse temperatures may have masked the development of further symptoms and because hops seldom show nettlehead until the third growing season after exposure to field infection (Thresh and Ormerod, 1971). However, in inoculation trials with purified virus preparations from nettlehead-diseased hops, it is usual for only some of the seedlings infected with AMV-H to develop nettlehead and for the others to remain symptomless (Thresh, personal communication). These apparent differences in sensitivity to infection have now been established in my work with Xiphinema and may be due to seedling variation. Alternatively, AMV-H and a second virus may be responsible for nettlehead, those plants infected with AMV-H or the second virus alone showing no symptoms. This possibility was first suggested by Legg (1961, 1964) who showed that a virus from symptomless Early Prolific hops which he named 'hop latent virus X' increased the sensitivity of Fuggle and Bramling plants to nettlehead, inoculated by grafting. Legg (1964) further demonstrated that the latent virus X caused line-pattern symptoms when grafted or sap-inoculated to American Neomexicanus types (Humulus lupulus L. var. neomexicanus

Nels. & Cockerell and its commercial derivatives, College Cluster and Keyworth's Midseason. He also obtained evidence that it was soil-borne and renamed it 'hop line-pattern virus' (HLPV). Subsequently, however, HLPV was considered a synonym to Bock's (1966) AMV-H (Martyn, 1968). Perhaps, the seedling Fuggle plant of unknown male parentage used in my test that showed line-pattern symptoms had Neomexicanus genes derived from males known to be growing in the area where the seeds were collected.

The hypothetical second virus has been designated 'N' by Legg (1964a). Its nature remains uncertain but it is definitely not necrotic ringspot virus (NRSV) as believed by Bock (1966), because healthy meristem and seedling hops that developed nettlehead in the field or after inoculation in the glasshouse, were shown to be free of NRSV when assayed on cucumber (Anon, 1967, 1968). Further, all the plants showing nettlehead or line-pattern symptoms in my transmission experiments were also free of NRSV. The fact that X. diversicaudatum transmitted nettlehead shows that the 'N' virus component (Legg, 1964a) is nematode-transmitted and it may be an unidentified NEPO-virus because purified concentrated preparations from some nettlehead sources now available contain only polyhedral particles when examined with the electron microscope (Thresh, personal communication). However, nettlehead preparations failed to react with antiserum to raspberry ringspot virus, strawberry latent ringspot virus, cherry leaf roll virus, tobacco ringspot virus, tomato ringspot virus (Bock, 1966). Further work is required to elucidate this point or alternatively to show whether nettlehead is caused by some but not all isolates of AMV-H.

The finding that AMV-H is seed-borne in hop is important to the researcher who must ensure that hop seedlings intended for experiments are virus-free. To the grower, this finding may be only of academic interest, since volunteer hop seedlings are usually weeded out early in the season during farming operations. Nevertheless, infected seedlings may still play a role, as they probably did in the past, in the production of infective X. diversicaudatum when they happen to grow in boundary hedgerows where they may live and multiply for years. While hop is a poor host for the nematode, the hedgerow plants, which consist primarily of hawthorn, blackthorn, bramble, wild rose, maple and grasses, are better (Pitcher and Jha, 1961; Thomas, 1970), hence, under this situation (i.e. hedgerows including seedling hops) multiplication and survival of both virus and vector are assured. Discounting the possibility that some of the true hedgerow plants are hosts of AMV-H, just as some are now known to be hosts of AMV-T (Thomas, 1970), these volunteer hops infected through seed may explain the reported prevalence of nettlehead along the sites of old hedgerows (Duffield, 1925; Keyworth and Hitchcock, 1943; Keyworth and Paine, 1950).

Tables 8, 15, 16 and 17, which show the host range of X. diversicaudatum and AMV-H, as well as the persistence of the virus in the nematode vector, indicate how both virus and vector may survive, multiply and spread in hop fields. In Table 8, where an evaluation of hop-field weeds as hosts for X. diversicaudatum was made in 90 ml clay pots, the differential effect of the various species on reproductive potential was already evident after a 10-week period.

Thomas (1970) cautioned that 15 weeks may not be sufficient to appraise fully the nematode's reproductive capacity due to its slow breeding rate (Flegg, 1968) and to the difficulty of synchronizing root growth of the various test species. In my experiments, such problems were apparently overcome satisfactorily using hand-picked, starved adult nematodes and by timing germination of the seeds so that the degree of root growth of each species was approximately equal when inoculated. Under these conditions, egg-laying on a suitable host such as Petunia hybrida was observed to be complete in 5-6 weeks, based on counts of larvae and uterine eggs.

The inference from my work is that X. diversicaudatum in hop fields multiplies primarily on weeds such as Stellaria media, Senecio vulgaris, Poa annua and Sonchus oleraceus, although the other weed species and hop may be involved in the maintenance and survival of both vector and virus. The practical significance of the four species of weeds found to be hosts of AMV-H, viz., Chenopodium album, Atriplex patula, Senecio vulgaris and Cirsium arvense, in the field spread of other diseases associated with arabis mosaic virus, needs to be assessed in future research. Nevertheless, the potential role of these weeds has already been demonstrated by the finding that AMV-H is transmitted through the seeds of C. album and A. patula, infected at seedling stage with hand-picked X. diversicaudatum, at an average rate of 54.6% and 30%, respectively. Lister and Murant (1967) reported 80% seed transmission of AMV-T in manually inoculated C. album, while in seeds of S. vulgaris collected from mature plants in the field, Taylor and Thomas

(1968) found 2% seed transmission. In my tests on C. album sap-inoculated with AMV-H at seedling stage, I obtained an average seed transmission rate of 66.7%. It is not yet known whether AMV-H is also seed-borne in S. vulgaris or in C. arvense; however, among the four new weed hosts found, S. vulgaris proved most suitable for the nematode vector and has seeds equipped with better means of dispersal than either C. album or A. patula. Although C. arvense produces few seeds, it is perennial and, like hop, both virus and vector can persist in or on the roots during winter fallow or whenever other suitable hosts are not available.

In addition to the various means of survival already discussed, AMV-H was found to persist in X. diversicaudatum kept without plants for 36 weeks in adults and 29 weeks in larvae. The mechanism of virus retention was suggested in electron microscope studies of nematodes fed on AMV-infected hops by Taylor and Robertson (1970) to be due to specific adsorption of the virus particles on the cuticular lining of the oesophagus, in contrast to the transient retention of raspberry ringspot virus and tomato black ring virus in Longidorus elongatus, which is due to specific association between the virus particles and the stylet guiding sheath (Taylor and Robertson, 1969). On present evidence, the persistence of AMV-H in X. diversicaudatum is of longer duration than that reported on AMV-T by Jha and Posnette (1961) (30 days) or by Taylor and Thomas (1968) (112 days) or even that of grapevine fanleaf virus in X. index by Raski and Hewitt (1963) (4 months). However, they are consistent with the findings of Harrison and Winslow (1961), that populations of X. diversicaudatum remained infective

with AMV-T for 8 months when cultured in soil containing Malling Jewel raspberry plants immune to infection.

Although sap-inoculation failed, AMV-H was successfully transmitted to Fragaria vesca and Petunia hybrida by X. diversicaudatum. However, repeated attempts to infect other known hosts of AMV-T, viz., S. media, P. annua, Taraxacum officinale and Matricaria matricarioides (Taylor and Thomas, 1968) failed, as did two attempts to transmit AMV-T to hop. These differences in host range, symptomatology and serological reactions confirm the basic strain differences between AMV-T and AMV-H first shown by Bock (1966).

Apparently, the current commercial hop cultivars were bred and classified mainly on the bases of sensitivity to wilt (Verticillium albo-atrum) and the aphid-transmitted hop mosaic and lately on alpha acid content. Since AMV-H is common to bare bine, split leaf blotch and nettlehead diseases, all widespread throughout hop-growing areas in England, it might be well to give emphasis to breeding for resistance and/or tolerance to this virus and its vector, X. diversicaudatum. Total elimination of virus reservoirs is not feasible or practicable as an approach to control considering the extent of distribution of AMV-H, but it is possible to minimize the spread and multiplication of the virus and, hence, loss by (a) planting AMV-free stocks ('A plus'), particularly in new sites, and (b) efficient control of weeds, many of which have been shown to be more suitable hosts for the vector and in some of which the virus is seed-borne. In areas where the vector is absent but the

diseases are present due to inadvertent planting of infected stocks, future problems could be averted by roguing the diseased plants and replanting them with healthy stock. The use of soil fumigants to eliminate X. diversicaudatum in old infested sites seems promising, but whether it would be effective and economical in the long term, considering the heavy soil types on which most hops are grown and the depth (90 cm) at which the vector can still occur, remains to be established. Nevertheless, Pitcher and McNamara (1971) and Thresh and Ormerod (1971) have shown, in current trials at Headcorn and at Horsmonden in Kent and at Rosemaund Experimental Husbandry Farm, Herefordshire, that autumn application of a mixture of dichloropropene and dichloropropane (DD) at 50-75 gal./ac. gave good control of the nematodes and halved the incidence of virus diseases due to AMV-H.

PART II

TRANSMISSION OF RASPBERRY RINGSPOT VIRUS AND ARABIS MOSAIC VIRUS BY LONGIDORUS CAESPITICOLA HOOPER, LONGIDORUS LEPTOCEPHALUS HOOPER AND XIPHINEMA DIVERSICAUDATUM (MICOL.)

INTRODUCTION

Three species of Longidorus, viz., L. elongatus, L. macrosoma and L. attenuatus, are now well known as vectors of the 'English' and 'Scottish' strains of raspberry ringspot and tomato black ring viruses (Harrison, 1962, 1964; Taylor, 1962, Harrison, Mowat and Taylor, 1961). However, the related species, L. caespiticola and L. leptocephalus, have never been implicated as vectors. They were first described by Hooper (1961) in soil samples from strawberry, blackberry, cherry and grassland. Attempts to transmit tomato black ring virus with either nematode failed in spite of their occurrence in large densities in the soil (Van Hoof, 1966).

Although NEPO-viruses often share common host plants, nematode transmission appears to be characterized by marked specificity. Thus, Dias and Harrison (1963) failed to transmit, with Xiphinema diversicaudatum, grapevine fanleaf virus and grapevine yellow mosaic virus, both of which are serotypes of arabis mosaic virus transmitted by Xiphinema index. However, Sauer (1966) succeeded in transmitting two serologically distinguishable strains of tobacco ringspot virus simultaneously with single specimens of X. americanum. The same vector species also transmitted both the type strain and the serologically distinct grapevine yellow vein strain of tomato ringspot virus (Teliz, Grogan and Lownsbery, 1966).

More recently, Taylor and Murant (1969) successfully transmitted English strains of raspberry ringspot virus (RRV-E) and tomato black ring virus (TBRV-E) with L. elongatus in addition to the Scottish strains of both viruses with which the nematode is normally associated. Five other isolates of both viruses, including a Dutch isolate of RRV and a German isolate of TBRV, were also transmitted.

The ability to transmit two serologically unrelated NEPO-viruses by one nematode species was demonstrated by Lister (1966) using arabis mosaic virus and strawberry latent ringspot virus and X. diversicaudatum. Taylor (1967) obtained simultaneous transmission of RRV and TBRV with L. elongatus to Stellaria media, but not to Fragaria vesca, Brassica rapa var. White Globe and Lolium perenne. Fritzsche and Kegler (1967) reported 5-10% transmission of an isolate of RRV from sweet cherry infected with Pfeffinger disease by X. divorsicaudatum, in contrast to 60-80% transmission of a similar isolate by L. macrosoma. The experiments described here report (1) transmission of RRV-E by L. caespiticola and L. leptocephalus and their depth distribution in a hop soil, (2) transmission of RRV-E by X. diversicaudatum and persistence of the virus in the vector, and (3) simultaneous transmission of RRV-E and AMV-T by X. diversicaudatum and L. caespiticola.

MATERIALS AND METHODS

Viruses.- (1) Raspberry ringspot virus, 'English' strain (RRV-E) - stock culture originally isolated from blackberry var. Himalaya Giant, kindly supplied by Dr. C.E. Taylor, Scottish Horticultural Research Institute. (2) Arabis mosaic virus, (AMV-T) - isolated from strawberry, kindly supplied by Mr. F.J. Ormerod, East Malling Research Station. Both viruses were maintained in Petunia hybrida Vilm.

Nematodes and virus transmission.- L. caespiticola and L. leptocephalus were obtained from around roots of hops in a field at Horsmonden, Kent, where their distribution in the soil was studied by the methods described in Part I; X. diversicaudatum was obtained mainly from woodland soil at High Halstow, Kent, and some from the Horsmonden hop soil. Extraction of the nematodes was done using Flegg's dish technique (Flegg, 1967). Tests with representative hand-picked nematodes of each species inoculated to healthy petunia bait plants showed that they were non-infective. Infective nematodes carrying either or both viruses were raised on petunia grown in a 2:1 steamed soil-sand mixture in 20 ml clay pots with drain hole plugged with plaster of Paris. This was done by transferring the nematodes, collected after 24 hours extraction time for the sluggish Longidorus spp. or overnight for Xiphinema, in bulk to the petunia infector plants. After about 2 weeks, when the plants had become fully established, the two youngest leaves were sap-inoculated with each virus; in this way, the initial effect is minimized particularly with plants infected

with RRV-E, and the time before which severe systemic symptoms occur was extended, thereby enabling the nematode to feed longer on infected, but still unrotted, roots. A typical source plant used for rearing infective nematodes is shown in Fig. 1. In the case of the mixture of both viruses, the source plants were inoculated with RRV-E one week earlier than with AMV-T, in an attempt to create equal degree of infectivity with both viruses, particularly in X. diversicaudatum, where preliminary results showed that AMV-T was more readily transmitted. After 5 weeks of acquisition access feed, the nematodes were re-extracted, hand-picked and transferred to healthy petunia test seedlings in egg cups at the rate of 50 adults or larvae (L3, L4) per plant. All test plants were placed in a 'gravel-tray moist chamber' (described in the Appendix) and kept in the growth chamber at 14-18°C. The roots of the source plants were assayed on Chenopodium quinoa Willd., on which gel-diffusion serology was done later, to confirm the presence of the virus. After 3-4 weeks, the roots of the test plants were washed free of soil and nematodes, ground with Celite and inoculated to C. quinoa or P. hybrida indicators. The identity of the virus in systemically-infected plants was confirmed by gel-diffusion serology, using a 1:40 dilution of antiserum to RRV-S or AMV-T.

Persistence of RRV-E in starved X. diversicaudatum.- In view of the unusually high rate of transmission, a test on the persistence of the virus in the nematode was made in an attempt to obtain some indication of the mechanism of virus retention. Infective nematodes were produced on



Fig. 1. Systemic symptoms of RRV-E in Petunia hybrida growing in small clay pot used for producing infective nematodes.

P. hybrida grown in small clay pots. After 6 weeks of access feed, the source plants were cut below ground level to starve the nematodes; a check on these plants showed that practically all the roots were rotted after one week. The pots were embedded to the brim in moist sand and the soil in the pot was kept moist throughout the duration of the test. At each test interval, the nematodes were sieved, hand-picked and transferred to healthy petunia seedlings in egg cups. After a further 5 weeks the roots were washed free of soil and nematodes, ground with Celite and inoculated to C. quinoa. Controls consisted of (a) roots of healthy petunia and (b) petunia inoculated with infective, unstarved nematodes.

RESULTS

Vertical and horizontal distribution of L. caespiticola and L. leptocephalus in hop soil.- As shown in Tables 1 and 2, L. caespiticola was found at all sampling depths from 11-79 cm. However, peak populations, which were remarkably uniform at all three distances (0, 45, 90 cm) from the hop plant, occurred from 26-34 cm deep, where 36.7% of the total were situated. The 2.5:1 ratio of larvae to females was similar at all depths. Based on the total population in 180 samples taken at the various depths and distances, there were 9.6% more females than males; and 7.9% L1 and 46.7% L4 in September, when the samples were taken. In relation to distance from the hop plant, most of the nematodes occurred at 45 cm (39.4%), compared with 35.5% and 25.1% at 0 cm and 90 cm, respectively.

L. leptocephalus, with a population density half that of L. caespiticola, was also found at 11-79 cm deep. The highest number occurred at 56-64 cm depth, mainly because of an abnormally high count in one replicate (Table 3). The nematodes were most dense at the 90 cm distance (52.1%), compared with 22.4% and 25.5% at 0 cm and 45 cm from the hop plant. No males were encountered during the September sampling, but a few were found when the same area was sampled in December. The average ratio of larvae to females was 1.8:1; 30% of the females were found at 11-19 cm depth, nevertheless, this gave the lowest ratio of females to larvae, suggesting that a high mortality occurred due to more severe environmental fluctuations.

Table 1. Vertical and horizontal distribution of *Longidorus caespiticola* in soil under hop plants (Horsmonden, Kent, September, 1969)*

Depth (cm)	Distance from hop (cm)			Totals
	0	45	90	
11-19	4	6	4	14
26-34	10	12	11	33
41-49	7	11	5	23
56-64	6	4	1	11
71-79	4	1	1	6
Totals	31	34	22	

*Mean of 12 x 200 ml samples in 6 replicates.

Table 2. Population structure (%) of Longidorus caespiticola in relation to depth in hop soil (Horsmonden, Kent, September, 1969)*

Depth (cm)	Growth stage						Totals
	♂	♀	L1	L2	L3	L4	
11-19	1.3	5.4	1.2	0.4	0.2	8.1	16.6
26-34	4.6	8.9	2.4	0.8	0.7	19.3	36.7
41-49	4.4	5.8	3.4	0.7	1.7	10.4	26.4
56-64	2.7	2.6	0.9	0.3	1.3	5.7	13.5
71-79	1.8	1.7	0.0	0.0	0.1	3.2	6.8
Totals	14.8	24.4	7.9	2.2	4.0	46.7	100

*Based on total counts in 180 x 200 ml samples taken at 0, 45 and 90 cm distances from hop plant.

Table 3. Vertical and horizontal distribution of *Longidorus leptocephalus* in soil under hop plants (Horsmonden, Kent, September, 1969)*

Depth (cm)	Distance from hop (cm)			Totals	% of total population†
	0	45	90		
11-19	1	2	4	7	20.5
26-34	3	3	3	9	25.5
41-49	3	2	2	7	20.5
56-64	1	2	8	11	29.6
71-79	0	0	1	1	3.9
Totals	8	9	18		100

*Mean of 12 x 200 ml samples in 6 replicates.

†Based on total counts in 180 x 200 ml samples.

Symptoms of feeding by L. caespiticola and L. leptocephalus Petunia hybrida, Poa annua, Stellaria media, Senecio vulgaris and Fragaria vesca, grown in a steamed soil-sand mixture in 20 ml clay pots, were infested with hand-picked L. caespiticola to determine whether it produced root galls. Only P. hybrida was infested with L. leptocephalus. After an access feed of 5 weeks, the roots were washed carefully, stained in acid fuchsin-lactophenol (Goodey, 1963) and galled portions of the roots were selected under the binocular microscope and photographed in clear lactophenol solution contained in a solid watch glass (Fig. 2). Root galls induced by L. leptocephalus on P. hybrida were smaller than those caused by L. caespiticola. On P. annua, the galls produced by L. caespiticola were similar to those produced by L. elongatus (Thomas, 1969), but on S. media, P. hybrida and S. vulgaris, the root galls formed by X. diversicaudatum were bigger and differently shaped (See Part I).

Transmission of RRV-S by L. caespiticola.- Four separate experiments were made using P. hybrida as test plant and, in all, the nematode inoculum consisted of a mixture of adults and larvae. In Experiment 1, bait plants grown in ice-cube moulds were each infested with 40 nematodes and in Experiments 2-4 they were grown in egg cups and infested with 30 per plant. Table 4 shows that 80% transmissions were obtained in each of two tests; however, in Experiments 2 and 3 the transmission rates were low; this may have been due to the poor growth of the bait plants. Typical symptoms of infected plants from Experiment 4 are shown in Fig. 3. Transmission to F. vesca, P. annua and S. media were also obtained. All

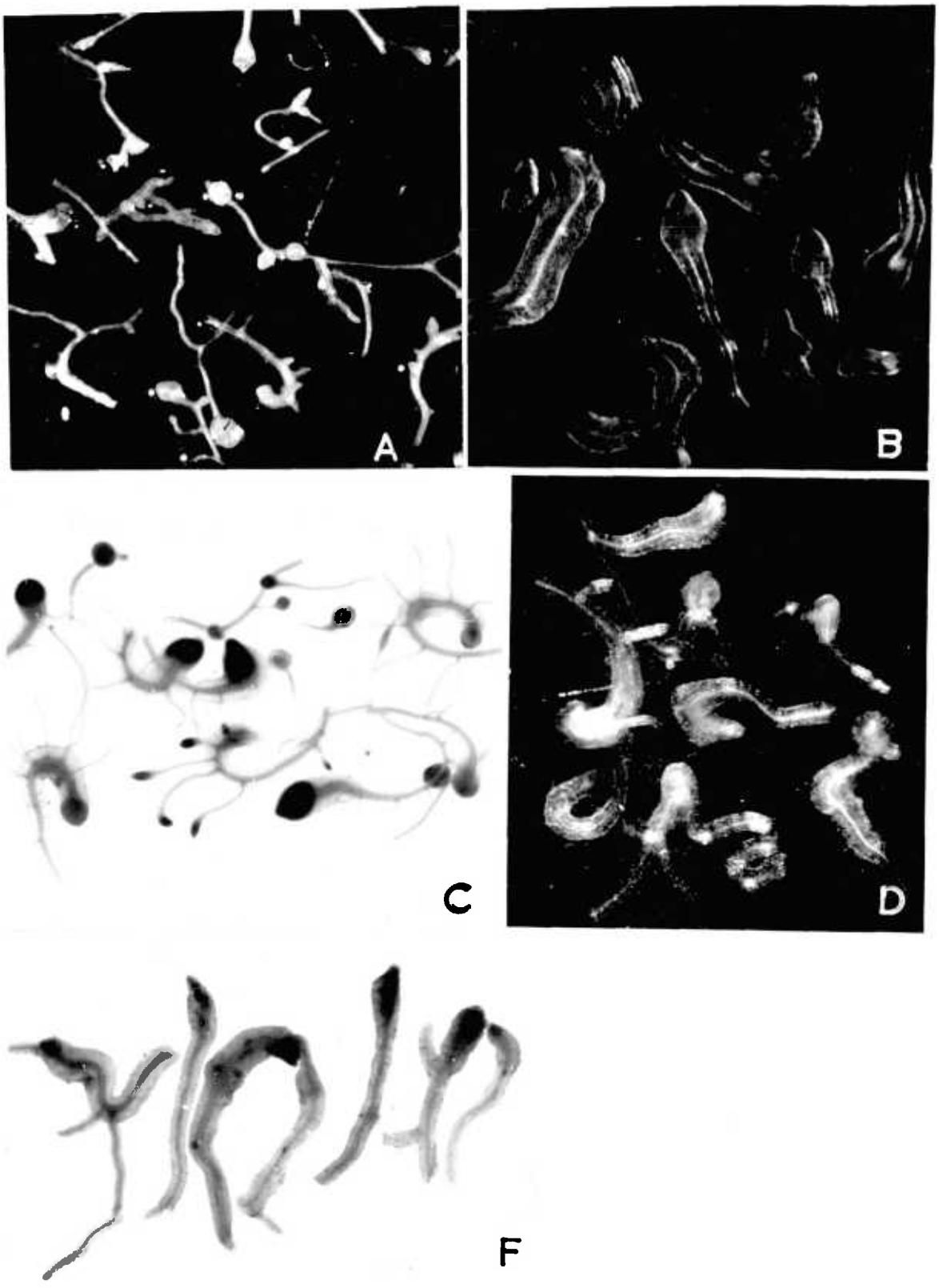


Fig. 2. Galls caused by Longidorus caespiticola on roots of (A) Petunia hybrida, (B) Senecio vulgaris, (C) Poa annua, (D) Stellaria media, (E) Fragaria vesca.

Table 4. Transmission of RRV-E by *Longidorus caespiticola*

Test plants	Transmission rate*
Petunia hybrida Vilm.	
Expt. 1	8/10
Expt. 2	2/10
Expt. 3	3/10
Expt. 4	8/10
Control (no virus, no nematodes)	0/38†
Control (no virus, with nematodes)	0/10
Fragaria vesca L.	3/10
Poa annua L.	4/8
Stellaria media (L.) Vill.	8/9

*Numerator is the number infected, denominator is the number of plants exposed to infection.

†Combined totals of four experiments.

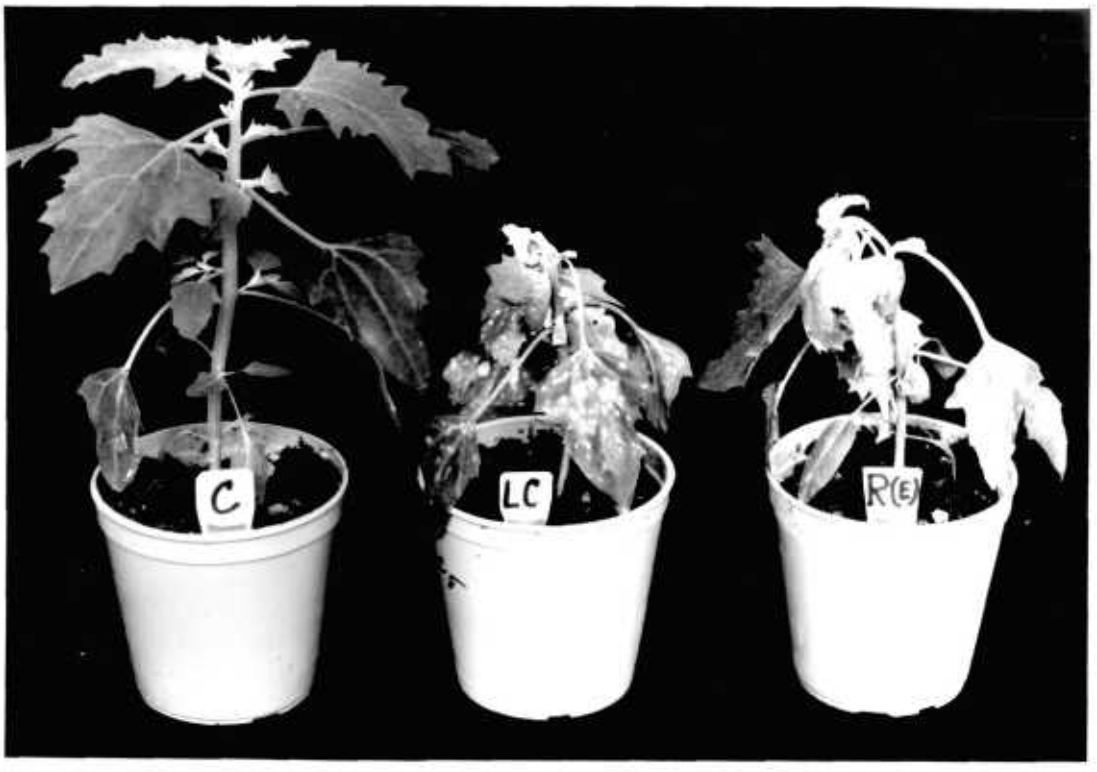


Fig. 3. Necrotic primary lesions and systemic infection caused by RRV-E on Chenopodium quinoa: (C) healthy control, (LC) transmitted by Longidorus caespiticola, (R-E) infected control, sap-inoculated with isolate of RRV-E.

were inoculated in small clay pots, using small rooted propagules of F. vesca or 4-leaf stage seedlings of the weeds, at 30 adults and larvae per plant. The roots of F. vesca were ground in phosphate buffer (1:5 w/v) to counteract virus inhibitors when inoculated to petunia (Jha, 1961). All the uninoculated control plants provided in each test remained healthy.

Effect of numbers of L. caespiticola on the transmission of RRV-E.- Table 5 shows 75% and 100% transmission at 15 and 30 nematodes per plant, respectively, but none occurred at one and 5 per plant. Apparently, no tests on the minimum number required to achieve transmission among previously known vector species of Longidorus have been published. However, Debrot (1964) obtained transmissions in 4 out of 6 plants inoculated with L. macrosoma at the rate of 20 per plant. Similarly, Fritzsche (1968) reported 48% and 75% transmission of Trespenmosaik virus by adult L. macrosoma at 20 and 50 nematodes per plant, respectively.

Transmission of RRV-E by L. leptocephalus.- Three transmission experiments, done in a similar manner as those with L. caespiticola, were conducted to determine whether L. leptocephalus could transmit RRV-E. In the first two tests, each with 8 plants and performed concurrently with Experiments 1 and 2 on L. caespiticola, the nematodes were transferred at 25 adults and larvae per plant; the results of both were negative. In Experiment 3, however, where 40 nematodes per plant were used, transmission occurred to 5 out of the 10 plants inoculated. The virus in the infected plants was checked by gel-diffusion serology.

Table 5. Effect of number of *Longidorus caespiticola* on the transmission of RRV-E

Nematodes per plant	Petunia bait plants infected out of 8
1	0
5	0
15	6
30	8
0	0

Transmission of RRV-E by X. diversicaudatum.- In Experiment 1, the drastic effect of virus infection reduced root growth, and when the nematodes were re-extracted and transferred to petunia at 60 per plant, only 18.2% were infected (Table 6). However, similar infective nematodes transferred at 50 per plant infected 70% and 80% of the test plants in Experiments 2 and 3, respectively. Adults gave 60% transmission and larvae alone (L3, L4) gave 100% transmission. The identity of the virus was confirmed in all infected plants by gel-diffusion serology. The roots of the C. quinoa indicators in Experiment 2 were ground with Celite and inoculated to P. hybrida and C. amaranticolor; typical systemic symptoms were produced in the former and characteristic local lesions were formed in the latter, thus confirming the results obtained from serology tests of the leaves.

Persistence of RRV-E in starved X. diversicaudatum.- All plants were infected by either adults or larvae at 50 per plant 16 and 26 days after cutting the tops of the source plants (Table 7). No transmission occurred after 40 and 51 days, indicating limited persistence of the virus in starved X. diversicaudatum. The control plants inoculated with roots of healthy petunia remained healthy while those infested with infective unstarved nematodes were all infected. The roots of the petunia infector plants were completely rotted within 16 days.

Simultaneous transmission of RRV-E and AMV-T by X. diversicaudatum and L. caespiticola.- In three experiments with X. diversicaudatum and in one experiment with

Table 6. Transmission of RRV-E by *Xiphinema diversicaudatum*

Expt. No.	Nematode		Transmission rate*	%
	Per plant	Stage		
1	60	AD,L3,L4	2/11	18.2
2	50	AD,L3,L4	7/10	70.0
3	50	AD,L3,L4	12/15	80.0
3	50	AD	6/10	60.0
3	50	L3,L4	8/8	100.0
Control	100	AD,L3,L4	0/10	0.0
"	0	-	0/30†	0.0

*Numerator is the number of *Petunia hybrida* bait plants infected, denominator is the number of plants inoculated.

†Combined totals of three experiments.

Table 7. Persistence of RRV-E in starved *Xiphinema diversicaudatum*

Starvation period (days)	Nematode stage	*P. hybrida* bait plants infected out of 8*
16	AD	8
16	L3,L4	8
0	AD,L3,L4	8
26	AD	8
26	L3,L4	8
40	AD	0
40	L3,L4	0
51	AD	0
51	L3,L4	0
0	AD,L3,L4	8

*All uninoculated check plants during each test interval remained healthy.

L. caespiticola, simultaneous acquisition and transmission of RRV-E and AMV-T were obtained (Table 8). All 48 petunia test plants became infected with AMV-T and 14.6% with RRV-E after infestation by X. diversicaudatum, indicating that the former was more readily transmitted than the latter. In the case of L. caespiticola, however, RRV-E and AMV-T were transmitted to 6 and 5 out of 8 test plants, respectively. In Experiments 1 and 2, RRV-E was not detected in serology tests of C. quinoa indicators in spite of apparently characteristic lesions. Further, 70% of the control plants inoculated with RRV-E alone were infected, suggesting that either the transmission or multiplication of RRV-E was reduced by the simultaneous presence of AMV-T. However, when the roots of the test plants in Experiment 1, left after serology, were ground with Celite and inoculated to P. hybrida, 2 out of 8 plants developed symptoms of RRV-E, but only after about 3 weeks when the symptoms of AMV-T became masked; gel-diffusion serology done on these 2 plants gave positive reactions to both viruses. In Experiment 3, where the ability of adults (AD) or larvae (L3, L4) of X. diversicaudatum to transmit both viruses was tested, 10% and 20% of the test plants were infected with RRV-E by adults and larvae, respectively; both adults or larvae infected all the test plants with AMV-T.

Table 8. Simultaneous transmission of RRV-E and AMV-T by *Xiphinema diversicaudatum* (Xd) and *Longidorus caespiticola* (Lc)

Expt. No.	Vector†	No. per plant	Transmission to petunia bait plants*		Indicator for testing bait plants
			RRV-E	AMV-T	
1	Xd	50	0/10	10/10	*C. quinoa*
1+	Xd	50	7/10	-	*C. quinoa*
1+	Xd	50	-	10/10	*C. quinoa*
2	Xd	50	3/10	10/10	*P. hybrida*
2	Xd	50	0/8	8/8	*C. quinoa*
2	-	0	0/10	0/10	*P. hybrida*
3	Xd (AD)	50	1/10	10/10	*P. hybrida*
3	Xd (L3,L4)	50	2/10	10/10	*P. hybrida*
3	-	0	0/10	0/10	*P. hybrida*
4	Lc	25	6/8	5/8	*C. quinoa*
4	-	0	0/8	0/8	*C. quinoa*

†Except Experiment 3, inoculum consisted of adults (AD) and 3rd and 4th stage larvae (L3, L4).

*Numerator is the number tested, denominator is the total number tested.

+Control: infective nematodes carrying either RRV-E or AMV-T only.

DISCUSSION

In 180 soil samples from a hop field, *L. caespiticola* and *L. leptocephalus* comprised 36.5% and 15%, respectively, of the total population of plant parasitic nematodes, in contrast to 48.5% of *X. diversicaudatum*. Peak populations of *X. diversicaudatum* occurred at 11-19 cm depth and alongside the hop; *L. caespiticola* at 26-34 cm depth at the 45 cm distance and *L. leptocephalus* at 26-34 cm depth at the 90 cm distance. These results show some similarities with the depth distribution of *L. elongatus* (Taylor, 1967) and of *L. macrosoma* (Fritzsche, 1968) where peak populations occurred at 0-30 cm and 10-30 cm, respectively.

Laboratory experiments have established, for the first time, that *L. caespiticola* and *L. leptocephalus* can transmit RRV-E. *L. caespiticola* appears to be the more efficient vector, as shown by transmission rates as high as 75% at 15 nematodes per plant, compared with 50% transmission with *L. leptocephalus* at 40 per plant. The potential of *L. caespiticola* as a vector was demonstrated in transmission tests on *F. vesca*, *S. media*, *P. annua* and *S. vulgaris*, all established hosts of *L. elongatus* and RRV (Taylor, 1967; Thomas, 1969). It is likely that *L. caespiticola* and *L. leptocephalus* also play a role in the dissemination of RRV through infected seed of their host plants as in *S. media* (Lister and Murant, 1967). Thomas and Taylor (1970) reported that *L. leptocephalus* occurred in 4% of 322 soil samples collected in Scotland, in contrast to 60% occurrence of *L. elongatus*; this may explain why the virus-vector potential of the former has not been noticed earlier. It

is now well known that Xiphinema and Longidorus spp. induce galling of plant roots. Also, there is evidence that galls formed by these two genera, in some plant species, possess characteristic sizes and shapes. Possibly this feature could be used for detecting them in the soil.

The results obtained on the transmission of RRV-E by X. diversicaudatum corroborate those of Fritzsche and Kegler (1967). However, the 5-10% transmission given in their paper contrasts with the higher rate in my tests. This may have been because different strains of viruses were used or because of the method of handling and inoculation. RRV-E has a persistent debilitating effect on P. hybrida, rendering subsequent leaves completely chlorotic, and highly infective nematodes were obtained by means of the small receptacle technique, wherein the nematodes were introduced first, followed by the virus. A reverse of this inoculation procedure, as in Experiment 1, or rearing them on P. hybrida planted in large volume of soil gave little or no transmission in three early trials. Stellaria media may be more suitable for rearing infective nematodes because its growth is unaffected by RRV-E and it is a better host for X. diversicaudatum.

RRV-E persisted in starved X. diversicaudatum for at least 26 days, compared with at least 42 days (Taylor, 1962) in L. elongatus. Taylor and Robertson (1969) showed that particles of RRV in L. elongatus are retained by specific association with the stylet guiding sheath whereas, when fed on plants infected with AMV only, particles have been found only in the cuticular lining of the buccal capsule.

The location of RRV in X. diversicaudatum remains to be established. Nonetheless, under field conditions, X. diversicaudatum may be capable of spreading RRV-E.

It has also been shown in this study that both X. diversicaudatum and L. caespiticola can acquire and transmit RRV-E and AMV-T, simultaneously, between petunia test plants. The results in Table 8 show that, in a mixed population, AMV-T was transmitted more readily than RRV-E by X. diversicaudatum. These confirm the results obtained in two preliminary experiments in which a culture of RRV-E on which X. diversicaudatum were being reared was inadvertently contaminated with AMV-T. Similarly, Bawden and Kassanis (1945) reported that simultaneous infection of a plant with two stylet-borne viruses can affect the concentration of one or both within the plant and that this is reflected in the level of transmission by aphids. This phenomenon was demonstrated in my results in Experiment 1 (Table 8) where 100% transmission of AMV-T occurred, but RRV-E was not detected serologically; however, in the controls, RRV-E alone and AMV-T alone gave 70% and 100% transmissions, respectively. Apparently, in mixed infection of petunia, the rate of multiplication of RRV-E is suppressed by AMV-T, hence, when gel-diffusion serology was done 8 days after inoculation in Experiment 1, only AMV-T was detected. However, when the roots of the indicator C. quinoa plants were inoculated to petunia, symptoms of RRV-E developed in two plants out of 8, 3 weeks after inoculation, when the symptoms of AMV-T became masked, indicating that a differential rate in virus multiplication exists and also

pointing to the value of using an appropriate indicator to detect virus transmission. The importance of a suitable indicator in detecting simultaneous transmission of two viruses by one nematode species was also shown by Taylor (1967) on RRV and TBRV by L. elongatus in Stellaria media.

On the one occasion on which it was tested, both RRV-E and AMV-T were transmitted from a mixed infection with almost equal efficiency by L. caespiticola.

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APPENDIX

A MICRO-CONTAINER TECHNIQUE FOR STUDYING VIRUS TRANSMISSION BY NEMATODES

INTRODUCTION

Since Hewitt, Raski and Goheen (1958) first showed Xiphinema index to be the vector of grapevine fanleaf virus, various workers devised different techniques in attempts to establish the role of nematodes as vectors of plant viruses. These methods, summarized by Raski and Hewitt (1963), range from growing healthy plants in field soil collected from around roots of diseased plants to inoculating hand-picked nematodes to bait plants in pots of sterilized soil; virus transmission being assayed later by inoculation of root sap to indicators. Jha and Posnette (1961) used 1-inch diameter glass specimen tubes containing 14-mesh autoclaved sand for growing petunia seedlings and studying the effect of duration of test feed by X. diversicaudatum in transmitting arabis mosaic virus. In 1967, Fritzsche critically examined the earlier methods and reported an 'improved standard technique' involving the inoculation of hand-picked nematodes in 4 x 4 cm 'solid watch glass' or glass block, containing just enough sterilized soil to cover the roots of 3-4 cm tall petunia test plants. Planted blocks were placed in a large Petri dish moist chamber, until the roots were ground and inoculated to indicator plants. This method proved unsuitable when adapted in attempts to transmit the hop

strain of arabis mosaic virus (AMV-H) using Chenopodium quinoa and hop. This appendix describes a modified version of this method, herein referred to as 'micro-container technique', and gives the results obtained in investigating transmission of the type strain of arabis mosaic virus (AMV-T) by Xiphinema diversicaudatum (Micol.).

MATERIALS AND METHODS

The solid watch glass (5 ml capacity) was used as the standard for comparing polythene ice-cube moulds (10 ml), plastic egg cups (20 ml) and small clay pots (20 ml). The drainage hole in each clay pot was plugged with plaster of Paris so that the nematodes could not escape. Infective X. diversicaudatum raised for 6 weeks on petunia that had been sap-inoculated with AMV-T were sieved out using the technique of Flegg (1967). The petunia bait plants used were first established for about 2 weeks by transplanting them singly at the 3-4-leaf stage, either in ice-cube moulds with small drain holes or in small clay pots containing finely sieved, steamed potting compost. When ready for inoculation, the soil was removed carefully and the root system washed gently in a dish of water so as not to damage the roots.

Hand-picking and inoculation were commenced after 3 hours of extraction time, thereby ensuring that all the nematodes were still very active. They were first collected at the desired rates in Perspex cavity blocks (Warner and Hague, 1960) and, with the aid of a finely-pointed bamboo splinter, they were then scooped into the centre of each micro-container containing about 1 ml of steamed soil-sand (2:1) mixture, with small amount of water. The roots of the bait plants were placed over the nematodes, covered with about 3 ml soil-sand mixture and watered carefully with the aid of a pipette. The micro-containers were partly embedded in moist sand contained in a 3" x 12" x 18" polythene 'gravel tray moist chamber' to reduce environmental fluctuations (Fig. 1). These moist chambers, filled with moist sand and



Fig. 1. Gravel tray moist chamber showing nematode micro-inoculation containers with Petunia hybrida. Left to right: small clay pot, polythene egg cup, polythene ice-cube mould, solid watch glass.

with a portable cover of 'Melanex' polyester sheet, were placed in a growth chamber at 14-18°C. There were 10 pots for each dosage rate and the treatments and the controls were randomized in 4 moist chambers. After 5 days, more soil was added in each micro-container to reduce the need for frequent watering, which was done from a polythene wash bottle with a fine nozzle. Soil moisture in the micro-containers was kept at approximately 20-25%; waterlogging caused rotting of the roots and loss of virus.

Only adult X. diversicaudatum were used in the test on the effect of number on virus transmission. No attempt was made to determine the effect of sex on virus transmission, although at the rate of one nematode per plant, 5 out of the 10 pots were inoculated with males and 5 with females. At the rate of 10 nematodes per plant, the inoculum consisted of 5 males and 5 females. However, in the test on recovery of the inoculum, 40 nematodes were used per pot, consisting of adults and third and fourth stage larvae. Recovery of the nematodes was done very carefully using Flegg's technique.

RESULTS

Three weeks after infestation, the roots of the petunia bait plants were ground in Superfloss Celite (Johns-Manville Ltd.) and inoculated to Chenopodium quinoa. Jha and Posnette (1961) reported 30% transmission with single adult X. diversicaudatum per pot using petunia test plants. Table 1 shows that at one nematode per plant, 100% transmission was obtained in solid watch glasses, 90% each in ice-cube moulds and egg cups, and 80% in clay pots. However, at 5 and 10 nematodes per plant, 100% transmission was obtained in all types of micro-containers; infection was more severe and uniform in all the plants and the symptoms, in general, appeared 1-2 days earlier than in the treatment with one nematode per plant, suggesting that the degree of infection is related to the number of vectors and, hence, the amount of virus inoculum. The results with one nematode per plant further show that males and females could transmit the virus equally. The virus in the infected plants was confirmed in gel-diffusion serology tests. All the control plants remained healthy.

The results in Table 2 show no significant differences between the number of nematodes recovered from the four types of micro-containers in either experiment. However, plants grown in egg cups and in clay pots had the most root and those in the solid watch glass had the least. The low number of galls produced in plants grown in clay pots, in spite of a big root system and with equally good nematode recovery, probably arose because the roots formed a mat around the sides of the pot and were thus rather inaccessible

Table 1. Effect of number of adult Xiphinema diversicaudatum on the transmission of AMV-T using micro-inoculation receptacles

Nematodes per plant	Petunia hybrida bait plants infected out of 10			
	Solid watch glass	Ice-cube moulds	Egg cups	Clay pots
1	10	9	9	8
5	10	10	10	10
10	10	10	10	10

Table 2. Recovery of *Xiphinema diversicaudatum* after 3 weeks from inoculation to *Petunia hybrida* in micro-inoculation receptacles

Receptacle	Nematodes recovered out of 40*				
	Expt. 1	Expt. 2	Total root wt. (g)	Mean galls/ plant	Root length (cm)
Solid watch glass	36.7	38.5	1.5	52.5	6.0
Ice-cube moulds	33.3	39.6	3.0	58.1	9.0
Egg cups	36.7	39.8	4.5	78.3	8.5
Clay pots	34.3	39.1	4.0	34.9	11.0

*Mean of 6 and 8 replicates for Experiments 1 and 2, respectively.

to the nematodes. By contrast, the roots of the plants grown in solid watch glass and in egg cups were shorter, so that the tips were more exposed to the nematodes, thus producing more galls in proportion to the amount of roots.

DISCUSSION

The techniques used in the transmission of soil-borne viruses by nematodes are varied, but the 'standard test' (Fritzsche, 1967) involving infestation with a single nematode species to test plants grown in pots, eliminated objections to the earlier techniques, viz., the possibilities of transmission by other nematodes or by root contact. The 'improved standard technique' devised by Fritzsche (1967) using solid watch glasses gave better feeding access to the test plants for the vector nematodes. However, the Petri dish moist chamber by Fritzsche proved too small for extensive tests and too shallow for fast-growing bait plants. The gravel tray moist chamber not only provided the necessary space and height, but the moist sand also served as an insulator to decrease temperature fluctuations, especially under conditions where temperatures were not controlled, by partly embedding the micro-containers. The sand in the trays should be sterilized between experiments to eradicate fungi and other contaminants. The use of these micro-containers and the gravel tray moist chamber proved highly successful not only in the transmission of AMV-T but also in the transmission of raspberry ringspot virus (RRV-E) by *X. diversicaudatum*, *Longidorus caespiticola* and *L. leptocephalus*.

When grown in egg cups or small clay pots to which soil can be added as the plants grow bigger, petunia can be grown for more than 3 months under moist chamber conditions, well beyond the 6-8 weeks reported by Fritzsche (1967). However, test plants that are badly weakened by moist chamber conditions such as C. quinoa or hop, successful transmissions of ANV-H by X. diversicaudatum have been obtained, and also of nettlehead disease of hop, by using either egg cups or clay pots partly embedded in sand without a moist chamber.

Production of infective nematodes has also been achieved successfully using the micro-container technique. This was done with hand-picked nematodes, but often it was easier to screen the nematodes in bulk and add them with small amount of water to petunia seedlings; the latter is especially suitable to egg cups or clay pots. Virus inoculation of infector plants is best done about 10 days after nematode infestation, when the roots have become fully established and feeding has begun. This is especially true of RRV-E or cherry leaf roll virus which cause severe damage to the plants. After 4-5 weeks, the nematodes are re-extracted by sieving. Since the soil-sand mixture leaves very little debris, hand-picking and infestation can begin about an hour after sieving. Water should be replaced in the extracting dish to allow all the nematodes to pass through; usually about 95% of X. diversicaudatum could be collected in 4 hours and about 95% of L. caespiticola in 16-20 hours.

The micro-container technique for virus transmission tests of nematodes has proved to be effective, gives relatively quick results and requires less space and materials than conventional techniques. Nevertheless, its success depends on using nematodes that are infective and active as well as in keeping the roots of the test plants vigorous and healthy.

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