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AN ASSESSMENT OF THE SOIL-SAMPLING DENSITY AND SPATIAL DISTRIBUTION REQUIRED TO DETECT VIRULIFEROUS NEMATODES (NEMATODA: LONGIDORIDAE AND TRICHODORIDAE) IN FIELDS

by
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Summary. Twin soil samples were collected from 130 sampling points from each of eight fields, during a survey of 37 fields in eastern Scotland, to determine the spatial distribution of virus vector nematodes. Samples were taken from 10 to 19 cm depth at each site from ten 100 m transects each 11 m apart with distances between samples of 5 cm to 51.2 m. Virus was detected by bait-testing field soil in 10 cm dia., 650 cm^3 plastic pots planted with *Petunia hybrida* and *Cucumis sativus* seedlings for four weeks. Symptoms in indicator plants, electron microscopy and the alkaline phosphatase version of F $(ab')_2$ ELISA were used for virus identification. Viruses were recovered from seven sites with combinations of two viruses occurring at four sites: raspberry ringspot and arabis mosaic (AMV), raspberry ringspot and tomato black ring, and tomato black ring and tobacco rattle (TRV) at two sites, and TRV alone at three sites. AMV was recovered from all 35 samples containing its vector, *Xiphinema diversicaudatum*, whereas with all other virus and vector combinations virus was not always recovered from samples containing vector nematodes and also the numbers of vector nematodes in samples did not influence the recovery of virus. The distributions of all viruses were highly aggregated and an analysis of all possible pairs of samples, classified by their distances apart, revealed a high probability for the recovery of virus from pairs of samples up to 8 m apart. Sampling for detecting nepoviruses and tobraviruses therefore should be based on a regular grid lattice with 7 m between sampling points.

Nepoviruses and tobraviruses are transmitted by longidorid and trichodorid nematodes respectively (Taylor and Brown, 1981; Brown, 1989). The viruses cause diseases in many crop plants, decreasing crop yield and/or quality. The viruses and their vector nematodes occur in most continents but are most prevalent in Europe and North America (Brown and Trudgill, 1989).

Since the first reports of nematodes transmitting viruses (Hewitt *et al.*, 1958; Jha and Posnette, 1959; Harrison and Cadman, 1959), field associations between viruses and their vector nematodes, including the horizontal and vertical distributions of the vectors, have been intensively studied in Europe (Boag *et al.*, 1989). Much information is availabile about sampling soil to detect virus vector nematodes (Cotten, 1979; Maas and Brinkman, 1980; Boag and Brown, 1985; Boag, 1986; Brown and Boag, 1986) and sampling plants to detect the presence and distribution of viruses (Barnett, 1986). However, there is a paucity of information about soil sampling for the detection of viruliferous nematodes i.e. those that are carrying and transmitting virus. We undertook detailed sampling of fields in eastern Scotland where nematode-transmitted viruses were suspected. The results are presented here and used to establish methods of sampling soil to detect viruliferous nematodes.

Materials and methods

In a survey of eight fields in eastern Scotland known or suspected to contain nematode-transmitted viruses, paired soil samples were collected from 130 points withim each field (Fig. 1a-d). Nematodes were extracted by a decanting and sieving method (Brown and Boag, 1988) from one of each pair of samples; the other sample was tested for nematodes transmitting virus. The soil samples were taken from between 10 and 19 cm deep. Distances between sampling sites were 0, 5, 10, 20, 40, 80 cm 1.6, 3.2, 6.4, 12.8, 25.6, 51.2 m in one direction, sampling in the next row was then repeated in reverse order in the opposite direction 11 m from the first row. Samples were taken from 10 rows in each field (Boag *et al.*, 1988). The field sites together with their associated nematode-borne viruses and vectors are given in Table I.

To detect nematodes transmitting virus, soil samples were bait tested (Cadman, 1956). Each soil sample was placed in a 10 cm dia., 650 cm^3 plastic pot and a single *Petunia hybrida* Vilm. seedling or, where only trichodorid nematodes were present, a *Cucumis sativus* L. seedling transplanted. The pots were maintained at 20°C in controlled temperature cabinets with lighting to provide a minimum 18 h day-length (Taylor and Brown, 1974). After four weeks the bait plant in each pot was washed free

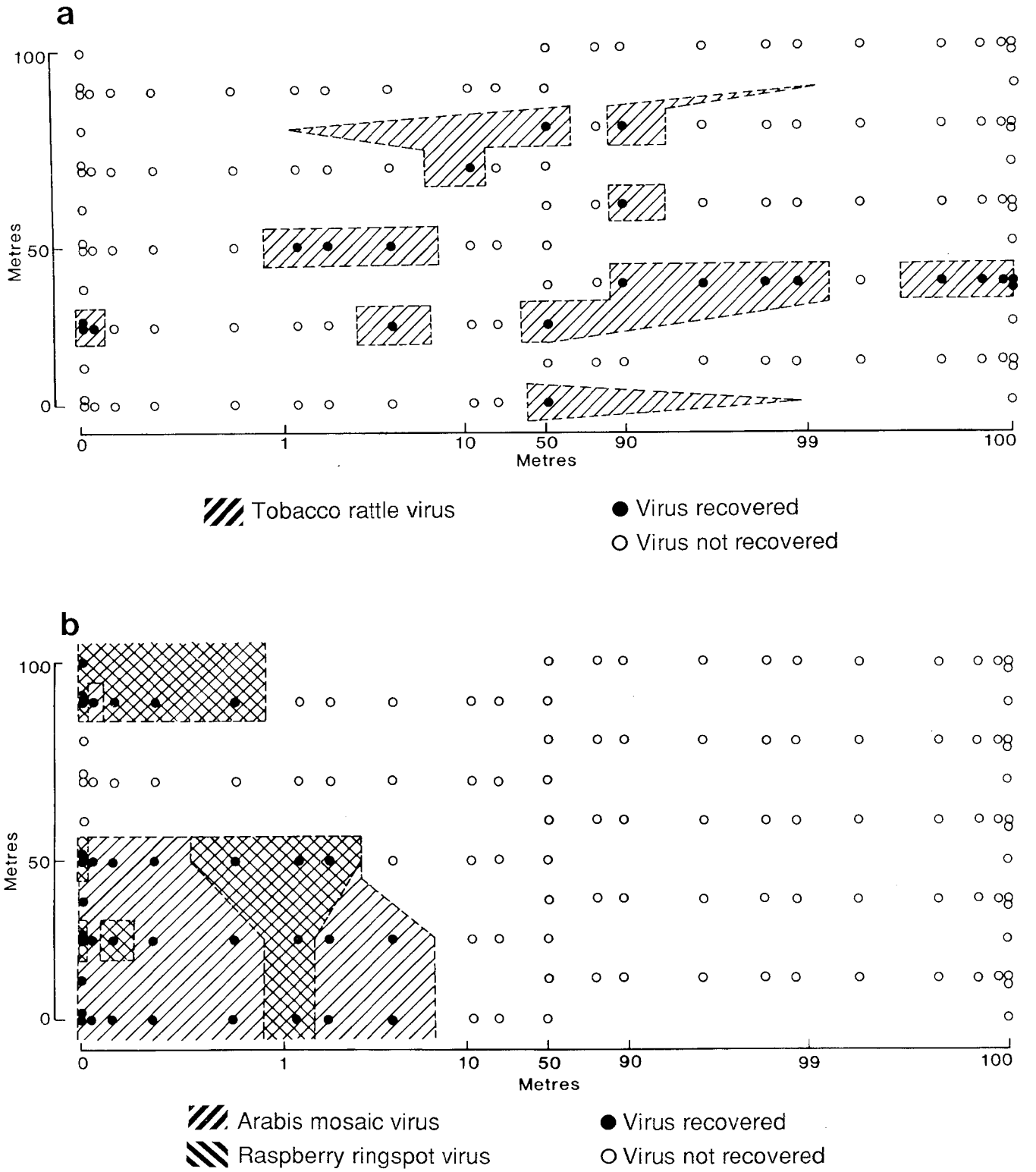
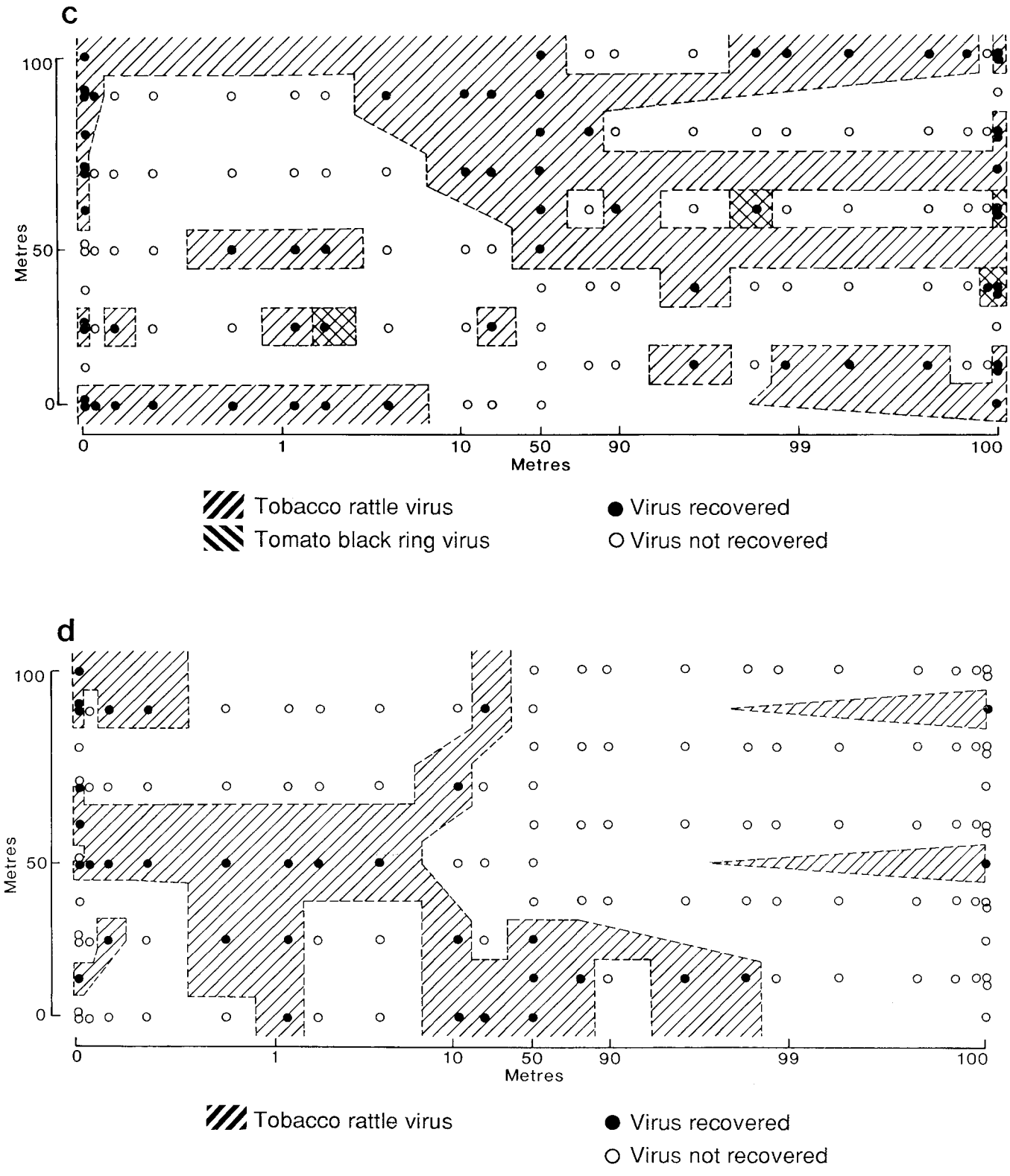


Fig. 1 - Diagrammatic representation of the distribution of nematodes transmitting virus at four field sites, plotted from the results of 130 bait tests taken from each site: a, Vicarsford; b, Hilton; c, Ingraston; d, Kinshaldy. Note that the direction of the log scale used for the Y axis should be reversed for each alternate transect.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Tobacco rattle virus
Tomato black ring virus
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Tobacco rattle virus
Virus recovered
Virus not recovered

from adhering soil particles and the roots comminuted. To detect nepoviruses, extracts made in phosphate buffered saline + Tween 20 + polyvinylpyrolidone, were tested for arabis mosaic, strawberry latent ringspot, raspberry ringspot and tomato black ring viruses by $F(ab')_2$ ELISA (Barbara and Clark, 1982). Protein A — alkaline phosphatase was used as conjugate and p- nitrophenyl phosphate as substrate. Reactions were stopped after 18 h with 3 M NaOH and the A_{405} values measured in a Titertek Multiskan 8-channel photometer.

To detect tobraviruses, root extracts were made in tapwater and the extract rubbed on leaves of *Chenopodium amaranticolor* Coste and Reyn plants, previously dusted with corundum. Tobraviruses induced necrotic lesions in the inoculated leaves three to ten days later. To confirm the presence of a tobravirus isolate, leaf extracts were examined for virus particles in an electron microscope.

The serotypes of the nepoviruses recovered were determined by Ouchterlony agarose-gel double diffusion serology designed to detect spur formation in comparisons with stock cultures of other isolates; tobravirus serotypes were determined by immunosorbent electron microscopy.

The distribution of nematodes transmitting virus and their degree of aggregation was examined by a method similar to that of Gray *et al.* (1986) but adapted to a sampling scheme not based on a regular lattice. All possible pairs of samples were classified according to their distance apart, and whether a particular virus was recovered from both, one or neither of the samples. Chi-squares were then calculated for each distance category to measure the degree of deviation from the distribution which would have been expected if the virus had been recovered at random. The direction of deviation was assessed by comparing the observed and expected numbers of double positives. The program, written in FORTRAN 77, can be used for up to 1000 data points which need not be constrained in rows or columns, and up to 1000 classes may be defined. The interval classes were chosen to give reasonable numbers in each class, depending on the frequency of infection, so that the values calculated would be reliable. An excess in the numbers observed over the numbers expected for pairs of samples which were both positive for virus indicated aggregation.

TABLE I - *Occurrence of* Longidorus, Xiphinema, Trichodorus *and* Paratrichodorus *virus vector species and their naturally associated viruses at eight field sites in eastern Scotland.*

Site	Crop	Nematode	Virus (serotype)	Reference
Tarvit, Fife	Raspberry	*L. elongatus* (de Man, 1876) Thorne *et* Swanger, 1936	Raspberry ringspot (Scottish) Raspberry ringspot (MX)	Cadman 1956 Jones *et al.*, 1989
Hilton, Fife	Pasture	*L. elongatus* (de Man, 1876) Thorne *et* Swanger, 1936 *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939	Raspberry ringspot (Scottish) Arabis mosaic (type - British)	Cadman, 1956 Smith and Markham, 1944
Ingraston, Tweedale	Pasture	*L. elongatus* (de Man, 1876) Thorne *et* Swanger, 1936 *T. primitivus* (de Man, 1880) Micoletzky, 1922	Tomato black ring (Scottish) Tobacco rattle (nc)	Harrison, 1957 Serotype not characterised
Blythe Bank, Tweedale	Swede	*L. elongatus* (de Man, 1876) Thorne *et* Swanger, 1936		
		T. primitivus (de Man, 1880) Micoletzky, 1922	Tomato black ring (Scottish) Tobacco rattle (nc)	Harrison, 1957 Serotype not characterised
Vicarsford, Fife	Barley	*P. pachydermus* (Seinhorst, 1954) Siddiqi, 1974	Tobacco rattle (nc)	Serotype not characterised
Kinshaldy, Fife	Pasture	*T. similis* Seinhorst, 1963	Tobacco rattle (PRN) Tobacco rattle (nc)	Cadman and Harrison, 1959 Serotype not characterised
Barry, Angus	Pasture	*P. pachydermus* (Seinhorst, 1954) Siddiqi, 1974	Tobacco rattle (PRN)	Cadman and Harrison, 1959
		T. cylindricus Hooper, 1962	Tobacco rattle (TC[B])	Brown *et al.*, 1989
Carnoustie, Angus	Barley	*P. pachydermus* (Seinhorst, 1954) Siddiqi, 1974	No virus detected	
		T. cylindricus Hooper, 1962	No virus detected	

Results

At the Carnoustie site, which had been previously well documented as containing tobacco rattle virus (TRV) and *(Para)Trichodorus* nematodes, virus was not recovered from any of the 130 bait tests despite the large numbers of trichodorids present in all soil samples. At all other sites virus was recovered in bait tests and the corresponding twin soil sample contained specimens of the appropriate virus vector nematode species (Table I).

At the Hilton site, there was a strong correlation between the presence of *Xiphinema diversicaudatum* and the recovery of arabis mosaic virus (AMV), but although *Longidorus elongatus* was present in all samples, raspberry ringspot virus (RRV) was recovered in only 16 of the 130 bait tests. These were concentrated in five areas, all from one half of the site (Fig. 1b). At the other five sites, virus vector nematodes were recovered from all paired samples but the presence of virus was not significantly correlated with the numbers of vector nematodes present in these samples (Table I; Fig. 1a-d). Two different viruses were present together with their vector nematode species at Hilton, Blythe Bank and Ingraston. Two serological variants of TRV occurred at Barry and Kinshaldy and of RRV at Tarvit (Table I; Jones *et al.*, 1989). The two TRV isolates present at Barry were found later to be transmitted by different *(Para)Trichodorus* species. However, as the different vector species and the virus isolates they were transmitting were not distinguished during the bait tests, data for analysis are recorded simply as trichodorids transmitting TRV. At Hilton, two isolates of AMV were detected which differed in their effects on *Chenopodium quinoa* Willd. test plants but were serologically indistinguishable (Jones *et al.*, 1989).

As a consequence of the pattern of soil sampling, certain distances between sampling points were represented more frequently than others and data on distances < 10 m was available only from samples taken along the transects. There was a marked aggregation in the occurrence of most nematodes transmitting virus, especially over short sampling distances (< 8 m), and the aggregation seemed to decrease at the distance where chi-squared values ceased to be significant (Fig. 2). When two viruses were present, the

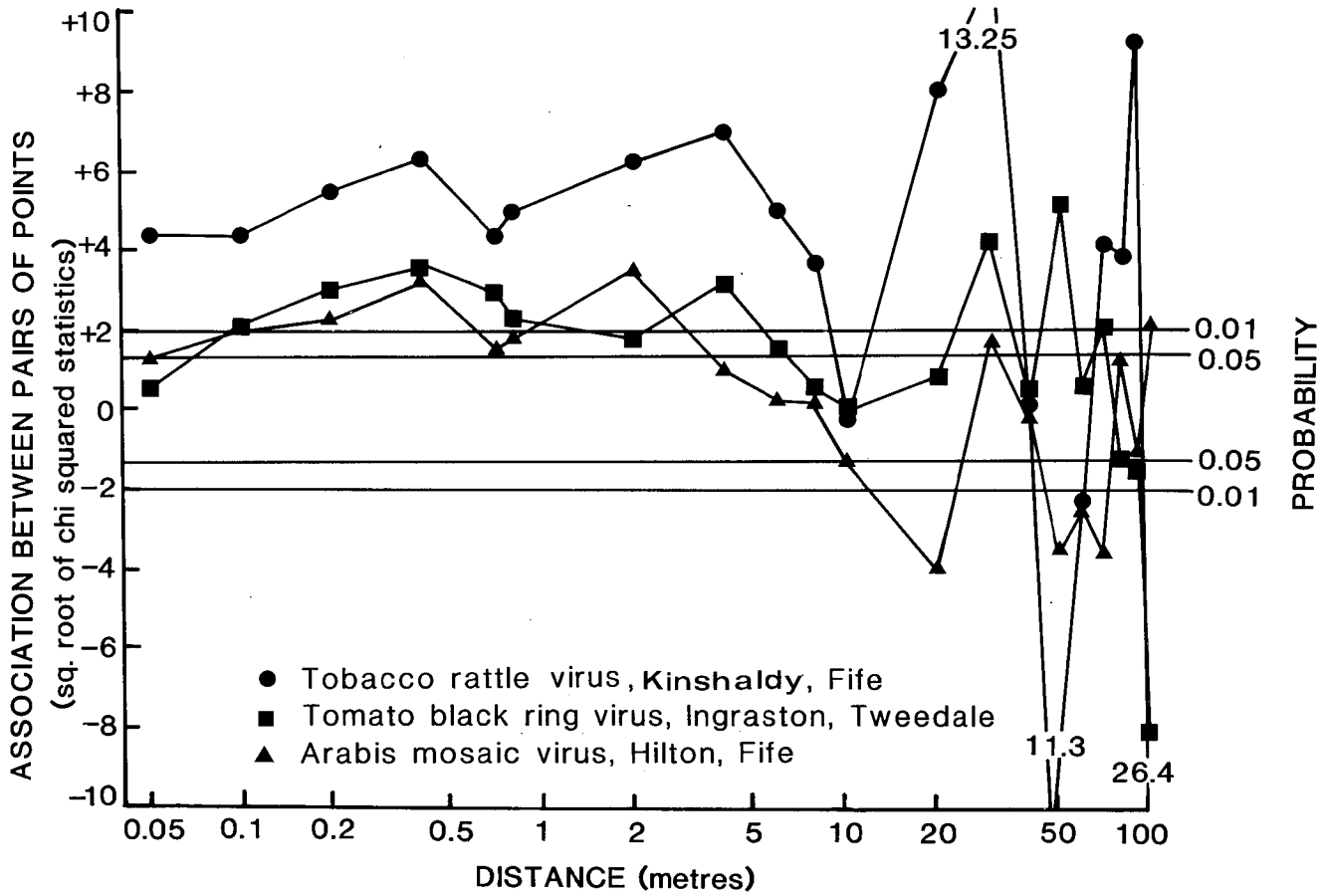

Fig. 2 - Association between pairs of sampling points from three sites from which nematodes transmitting virus were recovered in soil bait tests measured as the square root of the chi-squared statistic. The unit sign indicates positive or negative association. The 5 and 1% confidence intervals are shown as a guide to the probability of occurrence of virus positive pairings.

patterns were more complex with the chi-squared test usually indicating an excess of samples in which both viruses were present (Fig. 1b, and c). This suggested that aggregation of two nematode transmitted viruses may possibly reflect vector associations due to soil type or conditions.

For comparative purposes, test data sets were constructed with a random distribution of positive samples. In these the chi-squared values were generally small and of 84 equidistant pairings of sampling points only two were significant at the 5% level and one at the 1% level which was in agreement with the predicted values (Fig. 2). Only a small number of fields were examined, but the patterns of aggregation did not significantly differ. Overall the analysis of the association between equidistant sampling points indicated that up to distances of 8 m there was a high probability of virus positive pairings at the 1% or 5% levels (Fig. 2).

Discussion

The field distribution and spread of nematode transmitted viruses and the association with their vectors have been variously described (Harrison and Winslow, 1961; Pitcher and Jha, 1961; Meer, 1965; Taylor and Thomas, 1968; Converse and Stace-Smith, 1971; Uyemoto and Gilmer, 1972; Decraemer, 1980; McNamara, 1980). Virus distribution patterns in the field recorded by these workers were generally similar to those recorded at the sites examined during our study. Thus, the occurrence of a virus was associated with the presence but not with the numbers of its vector.

The occurrence of two or more distinct viruses at the same field site in association with their respective vector nematodes has also been reported previously (Murant and Taylor, 1965; Taylor and Thomas, 1968; Taylor and Brown, 1976) and the present study suggests that, at least in eastern Scotland, such combinations occur relatively frequently. Furthermore, the presence of variants of a virus at 3 of 7 sites suggests that this also occurs relatively frequently in eastern Scotland.

Failure to detect TRV at the Carnoustie site was unexpected as this site has previously been used for several field experiments to investigate the transmission of TRV and the chemical control of the vector *(Para)Trichodorus* nematodes (Cooper and Thomas, 1971; Alphey *et al.*, 1975). However, since the mid-1970s this site has had a rotation of cereal crops with the occasional crop of oilseed rape and potatoes. Possibly, the rigorous weed control regimes adopted with all crops grown at this site and the use of grain crops, which are not hosts for TRV (Barchend and Heidel, 1985; Gripwall, 1986) may account for the apparent eradication of TRV.

Soil sampling methods to detect vector nematodes have been described by several authors (Cotten, 1979; Maas and Brinkman, 1980; Barker and Campbell, 1981; Boag and Brown, 1985; Boag *et al.*, 1988; Boag *et al.*, 1989) but such methods may be inappropriate as alternatives to sampling for the detection of the viruses. Methods for sampling for viruses transmitted by nematodes have been described by Cotten (1979) and Antonova *et al.* (1982), and checking weed samples collected from field sites for the presence of nematode-transmitted viruses is suggested. However, as weed seeds may be wind dispersed, the presence of virus in weed plants does not provide unequivocal evidence for the transmission of the virus at the site from which weeds were collected.

Nematode-transmitted viruses have specific nematode vectors (Taylor and Brown, 1981; Brown *et al.*, 1989) and if the appropriate specific vector is not present the virus will not be transmitted to crops grown at that site.

Several methods for analysing spatial patterns of virus infected plants in field crops have been developed (Gray *et al.*, 1986; Madden *et al.*, 1987) but our approach was to adapt a geostatistical method used for predicting the occurrence of mineral-ore bodies. The semi-variogram of the geostatistical approach (Journel and Huijbregts, 1978) is widely used for continuous data and the present technique is similar in that it compares pairs of samples in distance categories, but it is distinct in using qualitative data. This method offers a novel and promising means for quantifying and interpreting aggregations but is not applicable to interpreting other spatial distributions. As used here the technique ignores the possibility that aggregation may vary along different axes. It would be possible to further sub-

TABLE II - *Theoretical probability of locating a single circular patch using a regular square grid sampling pattern.*

Distances between sampling points of a regular square grid (m)	Diameters of patch (m)				
	1	5	8	10	12
	0.79*				
2	0.20				
3	0.09				
4	0.05	>1.00			
5	0.03	0.79			
6	0.02	0.55			
7	0.016	0.40	>1.00		
8	0.013	0.31	0.79	>1.00	
9	<0.01	0.24	0.62	0.97	
10		0.20	0.50	0.79	>1.00
12		0.14	0.35	0.55	0.79
14		0.10	0.26	0.40	0.58
16		0.08	0.20	0.31	0.44
18		0.06	0.16	0.24	0.35
20		0.05	0.13	0.20	0.28

* Value 1 where patch is located and value 0 where patch will not be detected.

divide the distance classes to take account of direction but 130 sampling points probably are insufficient for this purpose. The statistical technique used here offers a powerful tool with which to identify patterns occurring at different scales i.e. patches of virus occurring at <8 m apart and at 70-80 m apart. This is done in much the same way as geostatistics (Journel and Huijbregts, 1978) or the block analyses of Grieg-Smith (1983) but with application to presence/absence data. As with mineral-ore deposits, virus patches are likely to be circular to elliptical with the longest axis following the direction of cultivation. Singer and Wickman (1969) have calculated tables of probability of detecting a target (mineral-ore deposits, patches of soil-borne virus, etc) with a specific size, shape/orientation when using a square, rectangular and hexagonal grid sampling procedure.

Results obtained during this study suggest that in fields in eastern Scotland patches of nematodes transmitting virus are generally 8 m or less in diameter. From the data in Table II it can be estimated that a 16 m regular square grid sampling pattern would give a probability of 20% of detecting such virus patches, an 8 m grid a 79% probability and that a 7 m grid would give a probability of detecting the virus patches $\simeq 100\%$. For high value crops such as foundation plantings of flower-bulbs or soft-fruits which are grown in relatively small areas within fields, a square grid sampling pattern with 7 m centres would be appropriate. With other crops, such as ware potato or commercial plantings of soft- fruits, a judgement is required by the extension worker in consultation with the grower as to the required level of probability of detection in relation to economic considerations.

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