SF/17

Verticillium wilt of strawberry: predicting disease risk

FINAL REPORT

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Location: HRI, East Malling

Abstract

Aspects of the application and further development of a soil test for predicting strawberry wilt risk were investigated. During the six months following introduction of the test technical problems at the ADAS laboratory carrying out the test were identified; there was no evidence of any seasonal effect in monthly and bi-monthly sampling at one site on the result of the test, although there was significant variability due to time of sampling; and a modified method of soil analysis which offered the promise of a more rapid and less expensive test proved to be unsatisfactory. The results obtained have already been exploited by ADAS in offering what is proving to be a useful and reliable advisory service for wilt control.

Summary

Research at HRI East Malling on verticillium wilt of strawberry has led to the development of a method for estimating the amount of the pathogen *Verticillium dahliae* in soil. Further work, partly funded by HDC and in collaboration with ADAS showed that this could be used as a test from which the risk of significant wilt in susceptible strawberries planted in the site could be predicted. This project, which covers the second half-years in 1991 and 1992, was concerned with aspects of the application of the test in commerce and possible improvements in the method of soil analysis.

The test was introduced commercially by ADAS in July 1991, and during the first six months of its application, sub-samples of soils analysed in the ADAS laboratory at Wolverhampton were analysed at HRI East Malling to check on operational reliability. One aspect of the test on which there was no information was the possible influence that the time of sampling could have on the result of the test. This was investigated by sampling an infested site at regular intervals from June 1990 to August 1991 and from February 1992 to November 1992. Finally, a modified soil analysis method in which the light, organic part of the soil is separated from the heavier mineral portion by flotation, and which offered the prospect of a more rapid and less expensive test, was investigated.

The results of the parallel analyses of 26 soils from July to December 1991 showed that there was a systematic underestimation at the ADAS laboratory which was found to be due to the soil extract used in the medium. There was no evidence of a seasonal

effect of sampling on the result of the soil analysis, but estimates of *V.dahliae* on samples taken at different times during the year varied significantly. This may be due to some short term influence of environmental factors on the germinability of pathogen propagules at the time of sampling, or to subtle and undetermined variability in the analysis procedure. The flotation method of soil analysis gave less than one percent of the recovery of *V.dahliae* obtained by the standard method and is clearly an unsatisfactory alternative. The reasons for the failure of this method are not understood.

Adjustments to the soil test procedures have been made in the light of these findings, and the follow-up of several cases in 1992 indicates that the advice given to growers based on the verticillium soil test during the winter 1991/92 has been generally reliable.

Introduction

MAFF-funded studies on strawberry wilt at East Malling in the late 1980s led to the development of a method of estimating the amount of the pathogen *Verticillium dahliae* in soil (Harris *et al.*, 1993). Based on the double premise that the soil was the most important source of infection (Keyworth & Bennett, 1951) and that there was a direct relationship between the amount of *V.dahliae* in soil and the amount of disease caused in strawberry as for other hosts (Ashworth *et al.*, 1972; DeVay *et al.*,1974; Evans & McKeen, 1975; Nnodu & Harrison, 1979; Grogan *et al.*, 1979), investigations were initiated to see if this soil analysis could form the basis of a means of predicting the risk of wilt associated with possible planting sites.

Financial support from HDC enabled a Chinese visiting worker to extend his stay at East Malling for a year (from May 1990 to July 1991) to assist with this work. The outcome of two years study was that the soil analysis appeared sufficiently sensitive and reliable to be used to predict wilt risk (Harris & Yang, 1990; Harris *et al.*, 1991).

The advisory possibilities of the soil analysis method had been appreciated at an early stage by ADAS, and several ADAS officers collaborated in the studies by providing soil samples and identifying sites. In 1990 an ADAS technician was trained in the soil analysis technique and several soils were analysed in parallel at HRI East Malling and at the ADAS laboratories at Wolverhampton. There was good agreement between the results at the two laboratories and the test was introduced to commerce by ADAS in July 1991.

During the first six months after introduction of the test, sub-samples of soils analysed at the ADAS laboratory were analysed at East Malling as a check. This work was supported by HDC and constitutes the first half of Project SF/17. An additional component of this project was to study the effect of time-of-sampling on the results of the soil analysis.

The second half of SF/17 was conducted after a six-month gap and was concerned with the continuation of the effect of sampling-time studies and possible improvements to the analysis method.

Although the soil analysis method is proving a significant advisory tool in dealing with strawberry wilt, it has some weaknesses. Firstly, it takes about five weeks to complete; this is because the propagules of *V.dahliae* require 3-4 weeks' incubation to produce recognisable colonies on soil plates. This should not generally be a problem as planning of strawberry plantings is usually done months in advance. A second and more serious problem is the cost, at £120 per sample. Most of this is the specialised labour which is required to examine the agar plate cultures after incubating the soil.

Another aspect of the analysis which could be improved is sensitivity. The sensitivity of the method is directly related to the amount of soil analysed, so it is theoretically unlimited. Unfortunately, the more soil analysed the greater the number of plates that have to be counted and the greater the cost. The maximum sensitivity of the method currently used is 0.1 units *V.dahliae*/g soil (counting 20 plates); and the critical threshold level for significant wilt in susceptible culttivars is considered to be about 0.5 units/g. However, for some highly wilt-conducive soils this threshold may be lower, and it would be useful to extend the sensitivity and the resolving power of the test at the lower levels of infestation. This can only be achieved by analysing more soil.

There are methods published in the scientific literature for enriching the amount of *V.dahliae* in soil. These depend on floating the fungus propagules away from the bulk of the more dense material by suspending soil in a solution whose specific gravity is greater than that of the propagules. Such methods offer the possibility of concentrating the *V.dahliae* from a comparatively large quantity of soil on to a small number of plates. This could simultaneously increase sensitivity and reduce the number of plates which have to be counted, thereby reducing costs. A flotation method employing saturated sucrose solution (Huisman & Ashworth, 1974) had been investigated earlier while the now standard soil analysis method was being developed (Harris *et al.*, 1993). In comparisons between the two methods, the standard method was generally more sensitive and gave higher counts by a factor of up to 12. A second flotation method reported to be effective for estimating *V.dahliae* in soil employed caesium chloride solution (Ben-Yephet & Pinkas, 1977). An evaluation of this method was carried out in the second part of the project.

Materials and methods

Comparison of soil analyses at East Malling and ADAS, Wolverhampton

From July to December 1991, 26 dried, sieved soil samples were received from the ADAS laboratory and analysed at East Malling

within from 1 to 38 days using the method described by Harris *et al*. (1993). When it became clear that the ADAS estimates were consistently lower than those of HRI, an experiment was set up to determine the source of the discrepancy. A soil which had shown a particularly large discrepancy was used. Soil suspensions and plates of the selective soil extract agar medium were prepared at the two laboratories and suspensions and plates were shared. The two suspensions were inoculated to the two media in all combinations and inoculated plates were shared again so that each laboratory had 1 plate of each of 8 combinations to incubate and count after four weeks.

Repeated sampling of infested soil

A 25 x 25 m plot of land at East Malling in a field which had been deliberately infested with *V.dahliae* in 1987 and which analysis in 1988 had shown to contain approximately 10 units of the pathogen/g soil was used for this work. The area was divided into 4 quadrants (A,B,C,and D) of 144 x 1 metre squares, separated by a 1m alley. At each sampling, a composite sample was taken from each quadrant made up of 10 single samples from randomly selected 1 metre squares. A new set of 10 was sampled on each occasion. Each individual sample was taken with a 20cm long, 2cm width cheese sampling tool.

Samples were taken at approximately 1 month intervals from June 1990 to August 1991. Samples were stored in an outside shed for various intervals of time from 1 to several weeks, and were then air-dried and sieved (2mm mesh), and stored in polythene bags in the shed until analysed.

Each sample was analysed as above except that the equivalent of 7.5g soil instead of 10g was plated out.

Because of the erratic results obtained from this series of samplings, a second, bi-monthly series was taken over the period February 1992 to November 1992. In this series, a standard protocol of processing and storing soil after sampling and before analysis was used to eliminate any variability from this source. Ten g soil were plated out but this was divided between the standard soil extract agar and a defined medium to see if the variability in the earlier work could be explained by medium batch differences. The moisture content of soil at the time of sampling was also determined.

The site was kept as weed-free as possible by cultivation during and between the two sampling periods.

Evaluation of a modified soil analysis method

This work was done using two soils known from the standard analysis to contain about 10 units of *V.dahliae*/g, one from East Malling and one from Suffolk. The method of Ben-Yephet & Pinkas (1976) which was used successfully by them for analysing loessial soils in Israel was evaluated with the East Malling soil. The method involves grinding a small quantity of soil and suspending

it in 1:1 w/v caesium chloride solution, shaking, separating the heavy deposit from the supernatant by using a separating funnel, recovering the material in suspension and plating this on to a semi-selective medium.

The method proved impractical because the relatively coarse East Malling soil blocked the funnel and good separation was not possible: no *V.dahliae* was detected. The method was modified to combine the potential of the flotation technique with elements of the proven standard method. Five g soil were dispersed by shaking
vigorously for one hour in 20ml caesium chloride solution. The slurry was allowed to sediment for 30 min and the supernatant was then removed by aspiration and washed through an 8μm pore-size membrane. The material on the membrane was resuspended in water and plated on to the soil extract agar medium. This method was used in a replicated comparison with the standard soil analysis technique.

Only about 1% of the colonies obtained in the standard analysis were obtained with the caesium chloride method. The reasons for the poor performance of this flotation method were not readily apparent. In the modified version of the published method the soil was in contact with the caesium chloride solution for a much longer period than in the original method. The authors of the original method claimed that the chemical was not toxic to *V.dahliae* but gave no details of how this was tested. Replicated experiments were set up to check the effect of caesium chloride solutions on microsclerotia of *V.dahliae*, comparing short and long exposure times.

In these experiments known numbers of microsclerotia of *V.dahliae* were shaken in two strengths of caesium chloride solution (1:1 or 1:3) for 3 or 60 min, recovered on sieves and plated on selective soil extract agar. The proportion of microsclerotia from each treatment germinating to produce recognisable colonies was determined. The microsclerotia used in this experiment were obtained from the stems of a potato plant that had died in the glasshouses at East Malling in 1992 after inoculation with *V.dahliae*. Microsclerotia 20-56μm in size were obtained largely free of plant material by sieving.

Results

Comparison of soil analyses at East Malling and ADAS, Wolverhampton

At East Malling, *V.dahliae* was found in all samples and the range was from 0.3 to 86 units/g soil (Table 1). In the ADAS analyses *V.dahliae* was not detected in 4 samples and the range of results was from 0 to 20.7 units/g. With one exception, the figures obtained at HRI were higher than those obtained by ADAS, and the ratio varied from 1.3 to 51. In nine cases the result would have had a significant effect on the advice given.

There appeared to be a problem that was causing a systematic underestimation in the ADAS tests, and this was investigated in a collaborative experiment. The outstandingly important factor contributing to the lower estimates proved to be the medium for the analyses at Wolverhampton (Table 2). Tests by ADAS indicated that the locally derived soil extract was the cause of the problem. The formula for a chemically defined recipe was communicated to the ADAS laboratory as an alternative to the soil extract agar. In earlier work at HRI this medium had been slightly less satisfactory than the soil extract agar.

The latest information from the Wolverhampton laboratory is that the medium problem has been overcome by using freshly prepared extract from the same local soil. In comparison with the defined medium at the ADAS laboratory, the soil extract agar gave slightly but consistently higher results. After processing more than 120 samples, the ADAS laboratory is now confident of the reliability and consistency of the method.

Repeated sampling of infested soil

The results over the first 14-month sampling period were very erratic (Figure 1). An analysis of variance on squareroot transformed data showed that there was no evidence for an overall change of pathogen levels in soil over the sampling period. However, there were significant differences between plots and between sampling occasions, although the latter were much more variable. The rapid fluctuation of estimates was unexpected and could be explained in several ways. The movements were too rapid to suggest seasonal variation and, in any case were not always consistent from plot to plot. It was considered that these fluctuations could be an artifact arising from the techniques employed. The survival of *V.dahliae* is reduced by a combination of warmth and moisture. It is therefore possible that the variable lengths of time and the different conditions (in polythene bags in an outside store) prevailing between obtaining samples and analysis could have contributed to the fluctuating results. Another possible source of variability was the soil extract medium used for analysis: although standardised methods of preparation are used, it is impossible to standardise the composition of the soil extract.

In the second phase of this work (February to November 1992), the results of analyses were again erratic and significantly so. There was a significant effect of the medium used but this was confined to the last of six analyses. On this occasion there was evidently a fault with the defined medium. Differences between plots were again significant although the time trends were generally similar for the four plots. The results shown in Figure 2 are for the soil extract medium. The full data are given in Table 3. These include figures for soil moisture at the time of sampling and after soil drying.

As yet there is again no evidence of a seasonal effect of sampling on the results of the soil test. It would appear either: 1) there are comparatively short-term changes in the

germinability of the propagules of *V.dahliae* (microsclerotia); or 2) the differences are caused by subtle and as yet, obscure variability intrinsic to the analysis method. The results of moisture determinations at sampling or after drying do not suggest that this could be a factor in determining germinability of microsclerotia (Table 3).

Sampling of this plot will be continued to see if any more light can be shed on the erratic results obtained in soil analyses.

Evaluation of a modified soil analysis method

Estimates of the amount of *V.dahliae* in two soils by two methods showed that the caesium chloride flotation method gave only a small fraction of that obtained by the standard method (Table 4), although the few colonies obtained were much larger than those obtained by the standard method.

Forty % of microsclerotia from diseased potato stems germinated after a brief exposure to water. Prolonged shaking in water increased the germinability of microsclerotia to about 60%. Exposure to caesium chloride (25%) had no effect on germination of microsclerotia of *V.dahliae*. Fifty % caesium chloride, the concentration used in the soil analysis, reduced germinability of microsclerotia to about half. This effect was similar regardless of how long microsclerotia were exposed to the chemical (Figure 3). This effect is much too small for toxicity of caesium chloride to explain the almost compete failure of the flotation method.

The poor performance of the caesium chloride method remains unexplained. It is possible that microsclerotia naturally occurring in soil are more sensitive to the chemical than the microsclerotia used in the experiments described here. Another possibility, that the majority of microsclerotia in the soil do not float in the caesium chloride solution, seems improbable. The sucrose flotation method, though also unsatisfactory, performed better than the caesium chloride method, yet the specific gravities of the solutions used are 1.4 and 1.6 g/cm^3 respectively. The specific gravity of *V.dahliae* microsclerotia is 1.3 g/cm^3 (Huisman & Ashworth, 1974).

Conclusions

1. The method of soil analysis for *V.dahliae* developed at HRI East Malling is reliable and, for most purposes, sufficiently sensitive to be used as a basis for predicting the risk of strawberry wilt associated with possible planting sites.

2. The technology has been transferred to the ADAS laboratory at Wolverhampton where, after some teething troubles, it is now being operated effectively as a service for wilt risk prediction for growers.

3. Experiments to investigate possible seasonal effects on the results of the soil analysis have been inconclusive. Significant

effects of time of sampling may be due to short-term non-seasonal changes in the germinability of *V.dahliae* propagules in soil or to subtle and as yet unidentified sources of variability in the analysis method.

4. The flotation technique of soil analysis employing caesium chloride solution proved unsatisfactory as an alternative to the standard method. The poor performance of this technique could be explained only in small part by toxicity of caesium chloride to microsclerotia of *V.dahliae*.

5. It would seem that any significant improvement in the soil analysis for *V.dahliae* will depend on the application of different technology such serology or DNA-hybridisation based techniques.

References

Ashworth, L.J., McCutcheon, O.D. & George, A.G. (1972). Verticillium albo-atrum: the quantitative relationship between inoculum density and infection of cotton. *Phytopathology* **62**, 901-3.

Ben-Yephet, Y. & Pinkas, Y. (1977). A cesium chloride flotation technique for the isolation of Verticillium dahliae microsclerotia from soil. *Phytopathology* **66**, 1252-4.

DeVay, J.E., Forrester, L.L., Garber, R.H., Butterfield, E.J. (1974). Characteristics and concentration of propagules of Verticillium dahliae in air-dried field soils in relation to the prevalence of verticillium wilt in cotton. *Phytopathology* **64**, 22-9.

Evans, G. & McKeen, C.D. (1975). Influence of crops on numbers of microsclerotia of Verticillium dahliae in soils and the development of wilt in southwestern Ontario. *Canadian Journal of Plant Science* **55**, 827-34.

Grogan,R.G., Ioannou, N., Schneider, R.W., Sall, M.A. & Kimble, K.A. (1979). Verticillium wilt on resistant tomato cultivars in California: virulence of isolates from plants and soil and relationship of inoculum density and disease incidence. *Phytopathology* **69**, 1176-80.

Harris, D.C. & Yang, J. (1990). Pre-planting prediction of strawberry wilt (*Verticillium dahliae*) risk as an aid in disease management. *Proceedings of the Brighton Crop Protection Conference Pests and Diseases* Vol. 1, 117-22.

Harris, D.C., Yang, J. & Locke, T. (1991). A practical approach to wilt risk. *Grower* **116**, 8-10.

Harris, D.C., Yang, J.R. & Ridout, M.S. (1993). The detection and estimation of *Verticillium dahliae* in naturally infested soil. *Plant Pathology* (in press).

Huisman, O.C. & Ashworth, L.J. (1974). Verticillium albo-atrum: quantitative isolation of microsclerotia from field soils. *Phytopathology* **64**, 1159-63.

Keyworth, W.G. & Bennett, M. (1951). Verticillium wilt of the strawberry. *Journal of Horticultural Science* **26**, 304-316.

Nnodu, E.C. & Harrison, M.D. (1979). The relationship between *Verticillium albo-atrum* inoculum density and potato yield. *American Potato Journal* **56**, 11-25.

Table 1
A comparison of soil analyses at two laboratories.

		Units of *V.dahliae* / g soil		
Sample code no.	Days to analysis	ADAS	HRI-EM	
Vic 3	1	0.5	0.6	
1309/A/91-1	6	17.2	53.2	
1309/A/91-2	6	1.4	3.5	
1309/A/91-3	6	0.4	3.1	*
1333/A/91-A	1	0.6	1.8	
1333/A/91-W	1	8.4	6.3	
275/C/91-FA	5	1.4	16.4	
275/C/91-PA	8	1.8	42.0	
275/C/91-TA	8	2.8	15.2	
273/C/91-1	8	0.2	5.6	*
273/C/91-2	12	0.7	3.8	
273/C/91-3	12	1.1	8.0	
1432/A/91-A	12	9.8	86.0	
1432/A/91-B	15	20.7	27.2	
286/C/91-A	5	0.1	5.4	*
286/C/91-B	5	0.3	1.2	*
274/C/91-1	15	0.0	2.3	*
274/C/91-2	15	0.0	2.4	*
274/C/91-3	19	4.8	24.2	
274/C/91-4	19	0.0	1.6	*
274/C/91-5	19	0.0	0.2	
1587/A/91-M	28	0.9	4.3	
1587/A/91-S	38	1.9	11.3	
1587/A/91-G	38	0.2	0.3	
1534/A/91-1	38	0.3	2.5	*
1534/A/91-2	38	0.2	1.7	*

* Significantly affecting advice to growers.

Table 2

Collaborative HRI-EM/ADAS experiment on the causes of discrepancies in soil analyses.

Units of *V. dahliae* / g soil.

	Source of difference				
	medium	soil suspension	plating	incubation and counting	earlier figures
ADAS	13.8	29.8	29.3	26.1	1.8
HRI	45.3	29.3	29.8	33.0	42.0

Table 3

Results of bi-monthly analyses of East Malling soil - composite samples from 4 plots.

Units of *V.dahliae* / g soil.

	Plots									
	A		*B*		*C*		*D*			
SOIL SAMPLE DATE	1 SEM	2 SDM	SEM	SDM	SEM	SDM	SEM	SDM	soil moisture	
									*	**
24-2-92	13.6	14.2	12.2	17.0	16.8	13.8	19.6	19.8	13.7	1.4
21-4-92	22.2	12.4	20.0	12.0	25.0	18.4	21.8	21.8	13.7	1.9
15-6-92	16.0	16.8	29.2	24.2	23.6	20.0	41.8	34.0	11.8	2.1
10-8-92	9.6	6.4	8.8	10.0	7.0	6.8	5.4	9.0	13.5	3.3
5-10-92	21.2	29.6	21.2	20.6	20.4	19.6	14.4	18.2	14.4	2.2
30-11-92	13.4	6.0	19.8	9.0	22.2	13.2	27.4	19.2	22.6	1.9

1 Soil extract medium

2 Selective Dox medium

* before drying

** after drying

Table 4

A comparison of caesium chloride flotation with the standard analysis for *V.dahliae* in soil.

Units of *V.dahliae* / g soil.

		Replicate				
Soil	Method	1	2	3	4	Mean
East Malling	Flotation	0.2	0.4	0.4	0.0	0.2
	Standard	10.2	15.8	16.2	19.2	15.8
Suffolk	Flotation	0.0	0.1	0.1	0.2	0.1
	Standard	15.2	15.5	14.8	16.3	15.5

Figure 1

Results of monthly analyses of East Malling soil - composite samples from 4 plots.

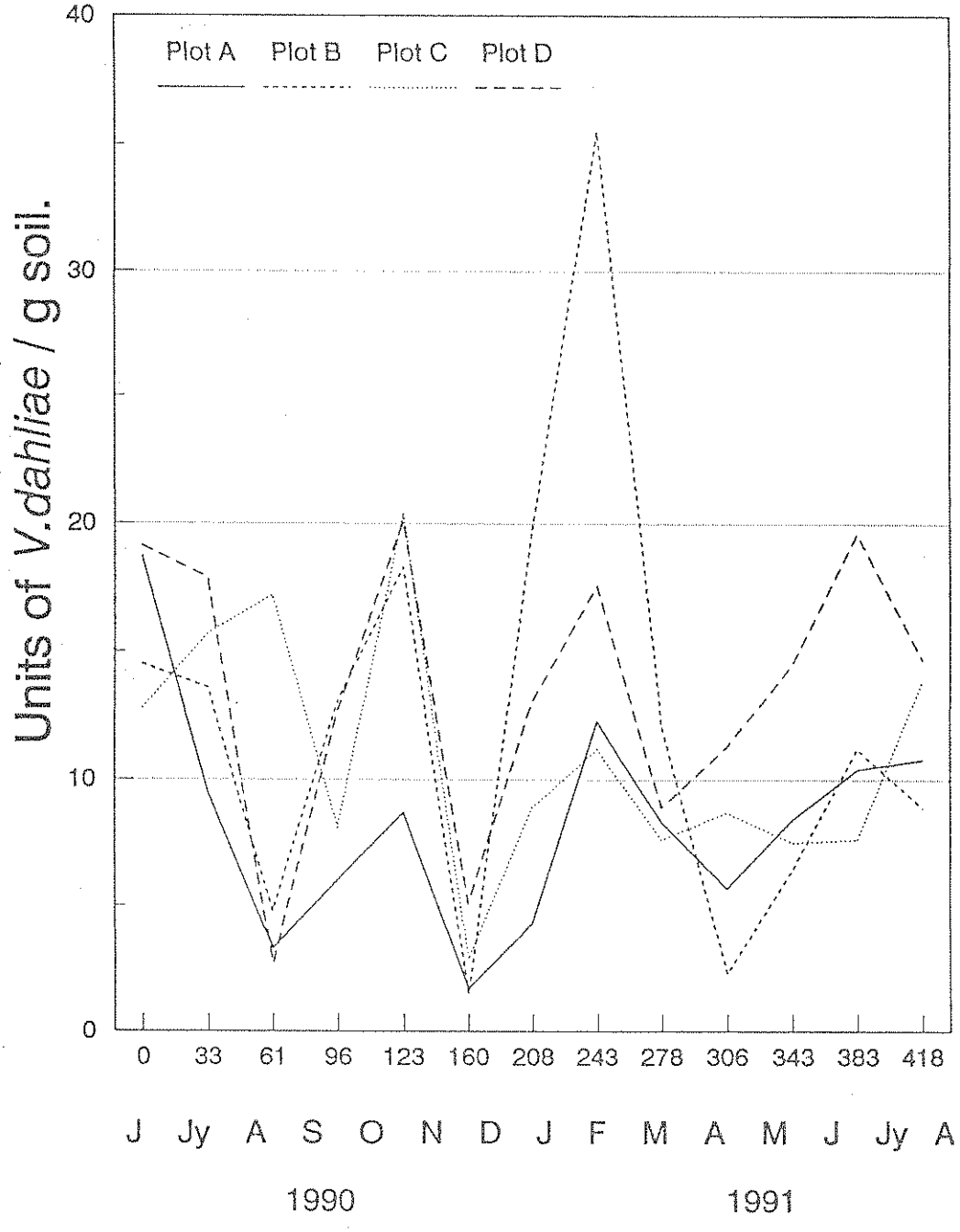

Figure 2

Results of bi-monthly analyses of East Malling soil - composite samples from 4 plots.

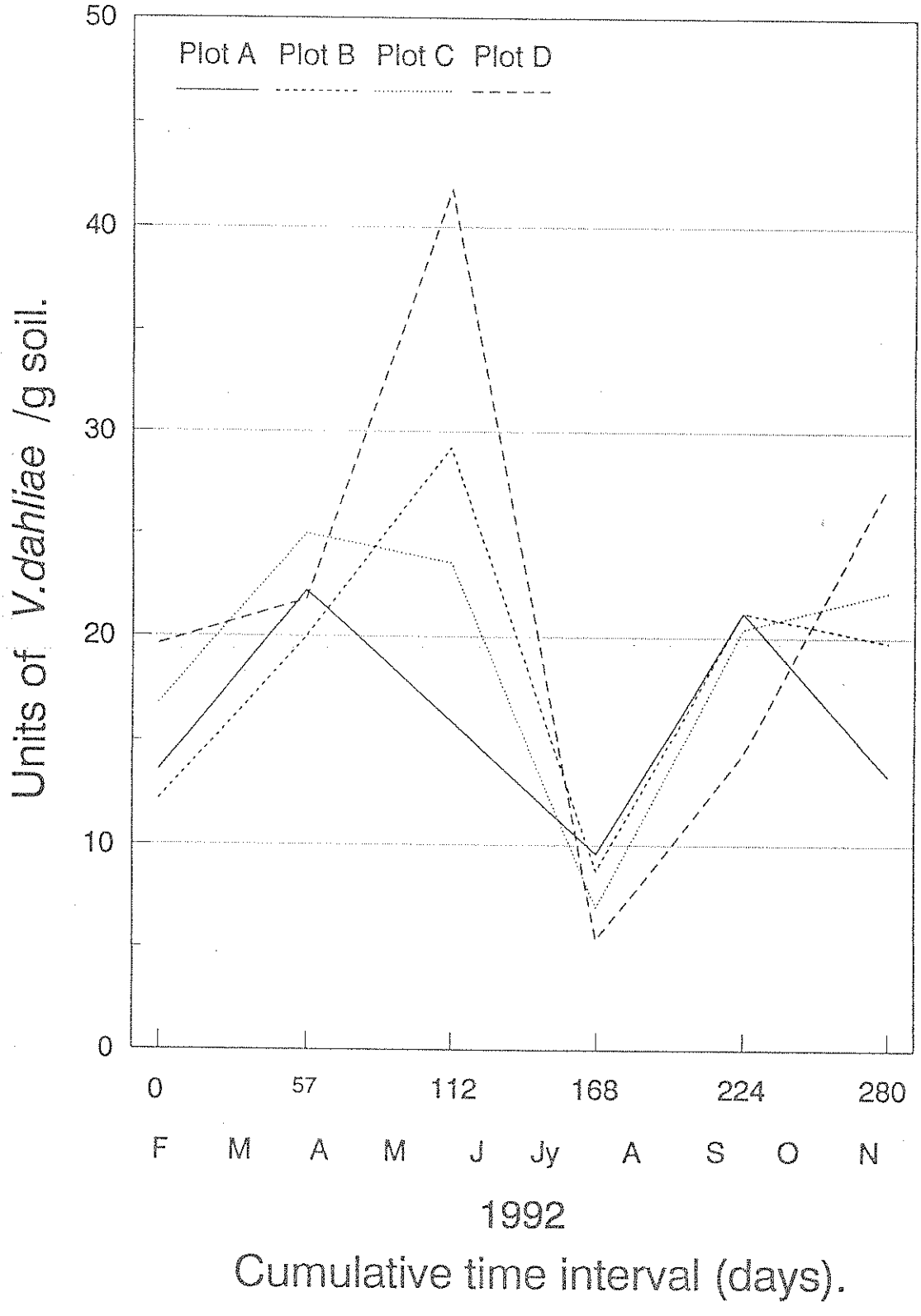

Figure 3
The effect of caesium chloride on microsclerotia of *V.dahliae.*