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Application of Remote Sensing Methods at Macroscopic and Microscopic Levels in Plant Pathology

Abstract. A review is given on the application of remote sensing and image analyzing methods in plant pathology, particularly at macroscopic and microscopic levels. The review illustrates various methods and techniques for detection, identification and assessment of symptoms of vegetation damage caused by diseases, pests and physiological stress, and for studies of the parasites as well as for use in demonstrations in plant pathology and related subjects and for training in conventional plant disease assessment.

Remote sensing is now widely used in order to identify, record and analyze soil and vegetation. There is an increasing interest to its application in plant pathology, for studies of damage caused by plant diseases and pests and physiological stress caused by pollution, water and nutrient problems. By remote sensing we study the multispectral signatures of a target area and its components. The electro-magnetic radiation from the object can be recorded and analyzed by optic-electronic instrument system. Multispectral signatures can also be recorded on photographic films, but the result is much dependent on the quality of the film, its grain size and resolution as well as its spectral sensitivity. By the use of suitable film/filter combinations and film types of various spectral sensitivity, however, we can get a good information on the multispectral signature of the target area.

Near infrared and infrared light is reflected deeper in the leaf tissue than visible light and may thus give complementary information on the physiological condition of the leaf and the plant. A leaf under stress is in many aspects also different from a normal leaf. Its size, shape, morphology and anatomy as well as its angle and thus its light absorption, transmission and reflection are different. A leaf under stress may close its stomata more or less which influences on its evapotranspiration. Such a leaf may have a slightly different temperature which may influence on germination of fungal spores on the leaf surface, the infection process and symptom development, etc. By infrared photography and spectroradiometry including thermography plant disease symptoms can be recognized and identified much earlier than they can be noticed by our eyes, and then the symptoms can be analyzed quantitatively and qualitatively.

From the above mentioned it is easy to understand that remote sensing has many applications in plant pathology and plant protection. Conventionally, remote sensing is referred to as a method to study a target area from high altitudes, from aircrafts and satellites. However, the technique and the methods can also be used at lower levels, over field experiments as well as at microscopic levels.

My work on remote sensing began in the 1950s when experiments were made to detect and identify previsual wilting symptoms caused by take-all and other root rots in cereals and grasses in field trials, and to study relationships between root rot severity, plant growth and yield. Remote sensing and image analysis were soon also found to be of very great interest and value in studies of leaf diseases, for early detection of the infection, studies of symptom development and to get increased accuracy in estimation of disease incidence - particularly as conventional disease assessment methods are based on visual estimation of the disease symptoms, leaf spots or root discolouration, etc. In modern research work on the development of models for plant disease prediction, calculations on epidemiology and crop disease loss assessment we need much improved and more accurate and reproducible methods to estimate the disease incidence. So far the primary data in disease incidence being put into the advanced models and computer systems have mostly been obtained by conventional disease assessment methods, such as visual estimation of percent leaf area infected by powdery mildew, rust or potato-blight, or scoring according to a provisional scale (0-1, 0-3, 0-4, 0-10, etc). Sometimes particular assessment keys have been used (such as those made by Clive James, 1971, or Anonymous, 1976), but also when we use these keys we have to make the estimations after visual examination.

The estimation of disease incidence can be very much improved by remote sensing and image analysis. In the literature there are now many reports which describe the use of such methods in field experiments, where the plant growth is recorded by hand-held cameras or spectroradiometers or where the instruments are mounted on a platform on a hydraulic crane (Fig. 1). However, these methods can also be applied at macroscopic and microscopic levels, and I will now briefly report on some such experiments carried out in Sweden and in cooperation with the Department of quantitative image analysis and instrument development at Ernst Leitz-Wetzlar GmbH, Wetzlar, West Germany, and some other departments.

The instrument system which I used mostly in these experiments consists of Leitz Texture Analysing System (Leitz-TAS) and some connected instrument systems. The image information is recorded via a TV-camera connected to the basic unit, the Leitz-TAS. The TV-camera can be furnished with macro-lenses for direct observation of the object or mounted on a macroscope or a light-microscope (Fig. 2). A computer and a printer were also connected and by its keyboard the operations of the instrument system could be programmed including printing out of the result. The basic unit, the Leitz-TAS, contains a very accurate microdensitometer, a TV-monitor and equipment for programming etc. The densitometer has capacity for recording and identifying spots of only 1% difference between the greylevels. 100 grey value intervals can thus be discriminated between the darkest grey value accommodated by the TV-system of the Leitz-TAS and the brightest point. I had also connected another instrument system consisting of a TV-monitor, microprocessors and an electric pen etc. By the pen it was very easy to define the area of the image to be analyzed, to select actual details to be studied, exclude those of less interest or artefacts etc. Several greyvalues could be simultaneously considered. A colour TV-display was also connected. This is not necessary for the measurements, but was found to be very practical as a complementary instrument. The image being analyzed was displayed in black-and-white on the two first-mentioned screens (Fig. 2:c,e), but in many cases it is not easy for our eyes to identify or differentiate images where the greylevel is only slightly different from the surrounding area. Such greyvalues can be translated to colours and displayed by the colour-TV in various colours or complementary colours (red-green, blue-yellow, etc). Signals of two image phases of different greyvalue can be simultaneously processed and displayed on

the colour monitor so that both image phases appear simultaneously in different colours. This also provides a control whether the two preselected grey value intervals overlap (mixed colour) or are exactly adjacent. This is a very handy and practical method which facilitates the work for the operator to a great extent during image analyses of disease symptoms or many other details in research. It seems also of great potential value for demonstrations as well as for training in such as disease assessment by conventional methods.

The above instrument system is easy to work with and easy to programme for various measurements. In a newer version the working monitor (Fig. 2:e) is improved and the operator can have his personal computer programs stored on microcassettes. A cassette is put into the taperecorder of the computer (Fig. 2:a) and the instrument systems can quickly be programmed for the actual routine work. Complementary program details can whenever easily be put into above main program for particular analyses, which makes the instrument system very flexible and easy to work with.

I used the Leitz-TAS for analyses of films (Kodachrome and Kodak Ektachrome Infrared films, etc) from field experiments in wheat with a complicated copper deficiency and water stress as well as various fungal infections (Fig. 3-4). The result of these image analyses of the films was highly correlated with differences in grain yield and other measurements in the field (Table 1). Similar very good result was obtained when photographs of field experiments on winter hardiness and winter damage in pasture and turf grasses were analyzed. In examinations of living samples of leaves infected by various fungal diseases such as powdery mildew, leaf rust and stripe rust, it was easy to calculate the leaf areas of various infections and the distribution of the lesions and their sizes, to discriminate between pustule area and surrounding halo areas or other zones which often can not be seen easily at conventional visual examination (Fig. 8) but may be of very great interest to investigate such as in studies on resistance and symptom development. When analyses were made of leaf spots and blotches caused by *Septoria* spp. it was easy to calculate the area of the leaf blotches and their distribution as well as the number or production of pycnidia. On potato tubers discriminations could be made between various types of scab and scurf as well as rots caused by *Fusarium*, *Phoma* and *Phytophthora* spp. etc and discolouration caused by various bacteria. When the TV-camera was mounted on a Leitz-Orthoplan light-microscope the image reading instrument system could be programmed to examine fungal spores, identify shape, calculate length, diameter and number of septa, discriminate between certain spore types and between germinating and not germinated spores, etc. as well as to examine effects of toxins and enzymes around infection spots. Analyses of chromosomes and various anatomical details can be made easily by the Leitz-TAS. At examinations of nematodes (Fig. 9) calculations were made on the body length and the diameter at various spots of interest along the body, the distance between those spots and the size of various organs. The procedure here was at first to make a mask of the image of the nematode and to project the original image within the mask, and then to point out by the electric pen the points of interest. The instrument can be programmed to record x/y-positions of actual spots and then make desired measurements. By the new version of the Leitz-TAS mentioned briefly above a lot of the measurements can be carried out automatically or semiautomatically. It is apparent that this instrument system has a very great interest and value also in taxonomy. When I examined leaves, mostly of clover, alfalfa, beans and tobacco, infected by various virus diseases it was possible to discriminate between various mosaic patterns etc. Examination of electron-micrographs of virus particles gave a quick information on shape and size, etc. When we study fungal growth on nutrient agar in Petri-dishes we usually measure the

colony diameter in two directions - such work is time consuming and laborious, particularly on fast growing fungi. This work can be much facilitated by the use of Leitz-TAS furnished with a macro-lens. After calibrating of the instrument it was programmed to measure the area (in mm²) of the fungal colonies and to give information on growth zones produced during the day-time or in the night, etc. The total procedures exemplified above including printing of tables and histograms of the result were made in a very short time.

The above are only some examples of the experiments carried out on the application of remote sensing and image analyses in plant pathology and plant protection. Another image reading instrument system with a very high resolution and capacity of analyzing various films at the same time is being developed at Stockholm University. Projects are being planned for its application in studies of the above mentioned type. Other types of instrument system for quantitative image analyses have been developed by Zeiss, Bausch & Lomb, Cambridge Instruments (Quantimet) and others. An interesting device to identify and count automatically field-gathered insect samples by pattern recognition is reported by Ellington & Flachs (1979). Three visual parameters, size, shape and colour are recorded and calculated by the instrument which then identify and count the various species.

The possibility of discrimination between various plant disease symptoms in field experiments or at macroscopic and microscopic levels and then to display the images in different colours is of very great interest and value for various types of analyses. However, it is also of apparent interest and seems very useful for demonstrations of plant disease symptoms and symptom development etc in studies of plant pathology and during training in conventional disease assessment. In such an application, for instance, we can start the training in assessment of simulated disease symptoms of various size and shape displayed in black-and-white. Then we can change to colours of high contrast, and complete the series with a more difficult situation, such as light green spots on a green or blue-green background. The instrument continuously shows the "disease symptoms" as well as information on their size, distribution, number and percent "infected leaf area", etc.

The above instrument systems worked mostly with very high accuracy. However, it should be mentioned that we had sometimes some problems during the densitometry of common colour films. Different colour zones, such as green and yellow leaf areas, could give the same grey value, and their areas could not be differentiated by the instrument. However, some such problems can be managed by using monochromatic light or filters. A red filter, for instance, can be placed over the picture of a leaf. It makes the green area darker and the yellow area can be measured better. Even better, but more expensive, is to use different TV-cameras with different spectral sensitivity and with a much wider spectral sensitivity, particularly in the red and infrared zones, than the above used. A system with sensitive multispectral radiometers scanning over the target area would, of course, be of particular value. Other methods of interest are spectrozonal photography and colour additive instruments and other instruments for image enhancement.

Remote sensing is evidently of great value for plant disease detection, assessment and inventories from high altitudes, aircrafts and satellites. Similarly, it has an apparent value as complement to conventional field experiment methods. However, it is also apparent that remote sensing applied for use close to the object together with instrument system for quantitative and qualitative image analyses as exemplified above is of very great interest and value in plant pathology and plant protection and related subjects such as taxonomy, virology, mycology, microbiology, nematology, entomology, genetics and plant breeding, etc. The instruments above may be expensive. The methods and techniques can be improved. They open fascinating possibilities to improve research in the above mentioned subjects.



Fig. 1.

Cross-country lorry-mounted hydraulic crane used as instrument platform during the multispectral signature studies at Uppsala.

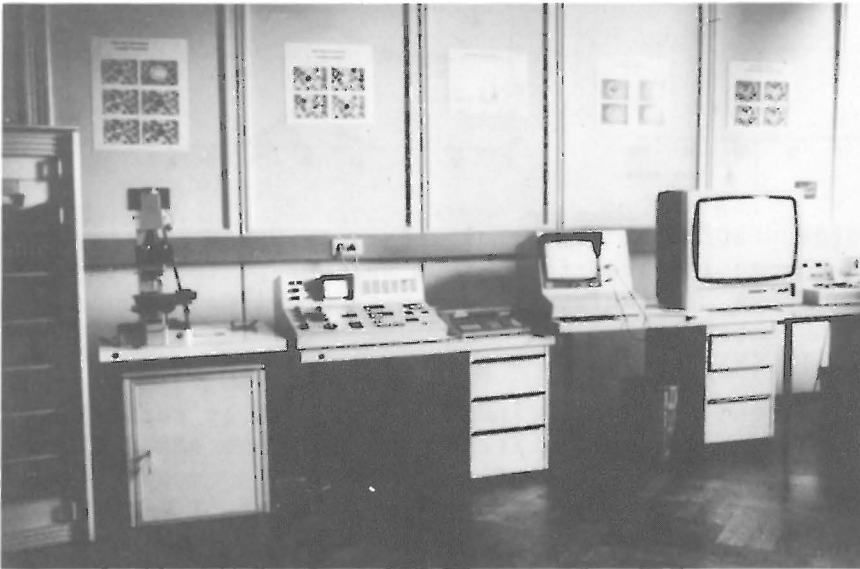


Fig. 2. Laboratory at Ernst Leitz-Wetzlar GmbH, Germany; Department of quantitative microscopy and image analysis, showing the Leitz-TAS and connected instruments used in some of the present investigations. From left to right:

- a = Computer with casset-tape recorder for programming, etc.
- b = Leitz Orthoplan microscope furnished with a TV-camera.
- c = Leitz-TAS basic unit.
- d = Teleprinter and keyboard for programming. A later version has also an x/y-line printer.
- e = Working monitor with electric pen for interactive programming.
- f = Colour-TV monitor.

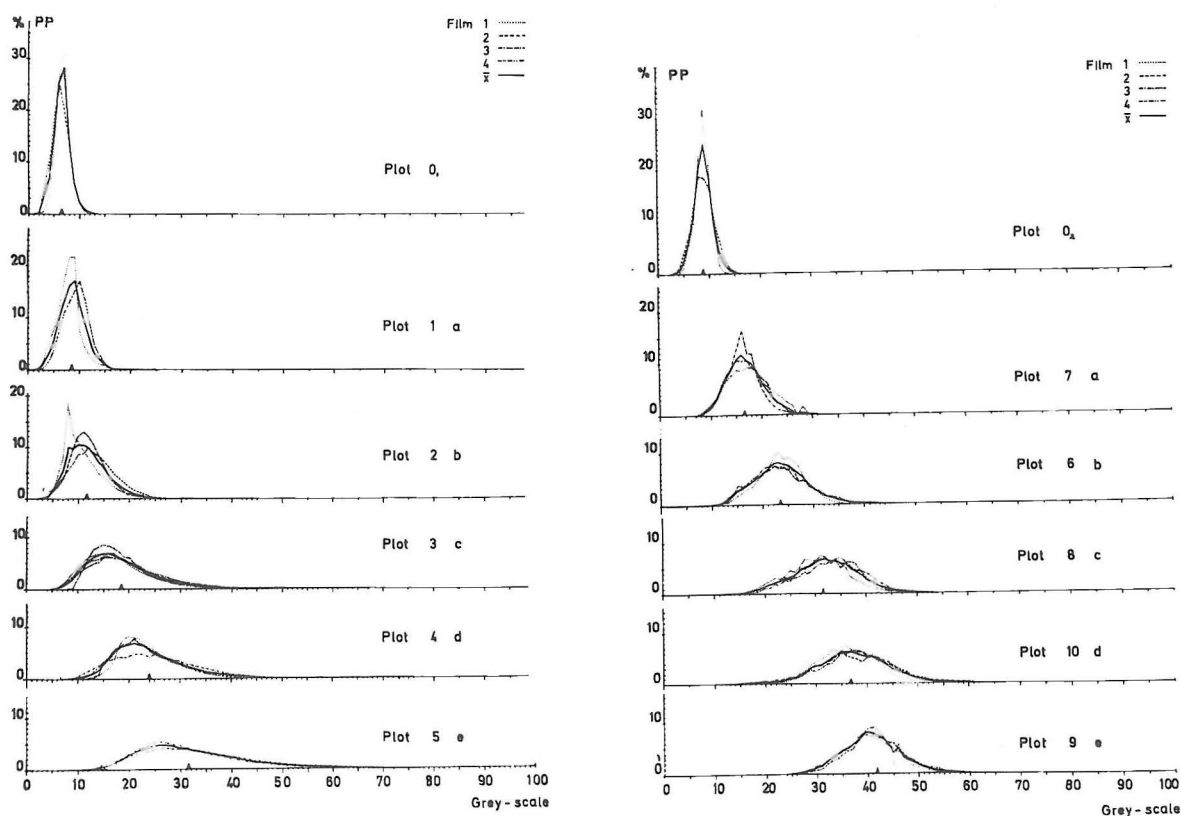


Fig. 3-4. Graphic representation of the result of Leitz-TAS densitometer analyses of Kodak Ektachrome Infrared films from various plots in a field experiment in wheat at Mbeya, Tanzania, in 1973 with previsual symptoms of copper deficiency and water stress. Y-axes = number of pixels (%) and X-axes = greyvalue (%).

Table 1. Relationship between grey-value index, calculated by Leitz-TAS analysis of the IR photographs (7 April; Feekes growth stage scale 6) and grain yield (18 July; Feekes scale 11.2) of the wheat field experiment mentioned in Figures 3-4.

Plot	Fertilization, kg/ha			Grey-value index of IR photographs 7 April 1973 (x , %)	Grain yield at harvest 18 July 1973 (y , kg/ha)
	N	P ₂ O ₅	K ₂ O		
0 o	0	(0)	(0)	6.50	-
1 a	0	60	40	8.49	1477
2 b	20	60	40	11.76	1352
3 c	40	60	40	18.46	768
4 d	80	60	40	24.00	175
5 e	160	60	40	31.49	400
0 o	0	(0)	(0)	8.75	-
7 a	0	120	80	16.66	931
6 b	20	120	80	23.22	863
8 c	40	120	80	31.55	176
10 d	80	120	80	36.78	21
9 e	160	120	80	40.19	18

Linear regression: plots 1-5: $y = 1885 - 55.77x$, $r = -0.9043$
plots 6-10: $y = 1747 - 45.34x$, $r = -0.9610$

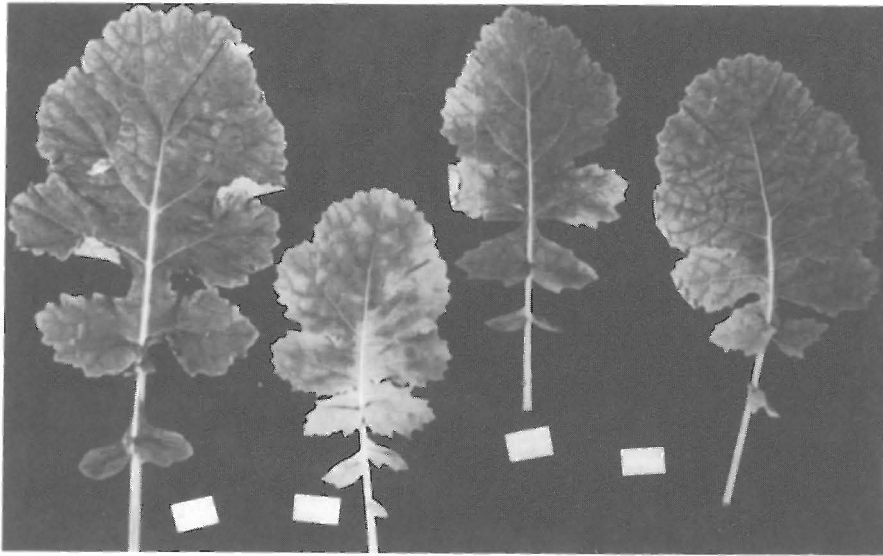


Fig. 5.
Copy of Kodak
Plus X film.

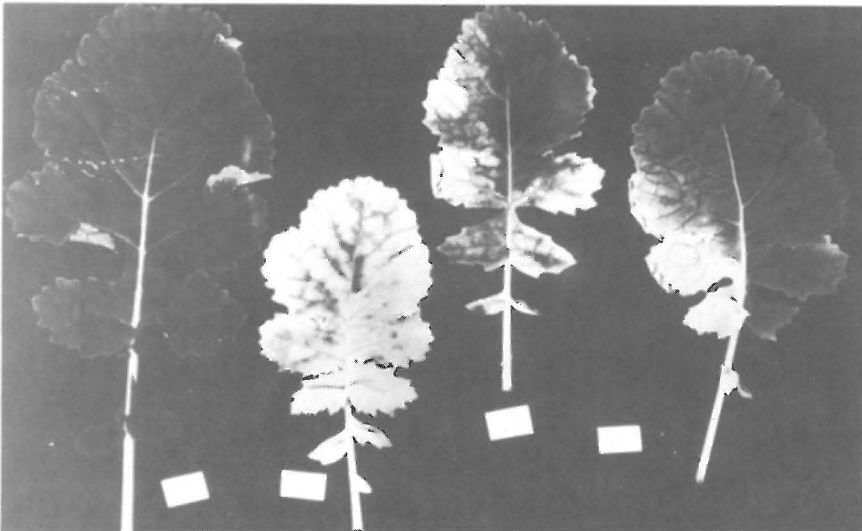


Fig. 6.
Copy of Kodak
Ektachrome
Infrared film.

Fig. 5-6. Leaves of oil seed rape, *Brassica napus* var. *oleifera* Metzger. To the left a healthy leaf. To the right three leaves infected by *Verticillium dahliae* Kleb. The wilting symptoms caused by the fungal infection are shown up more clearly on the IR film than on the Pan X film.

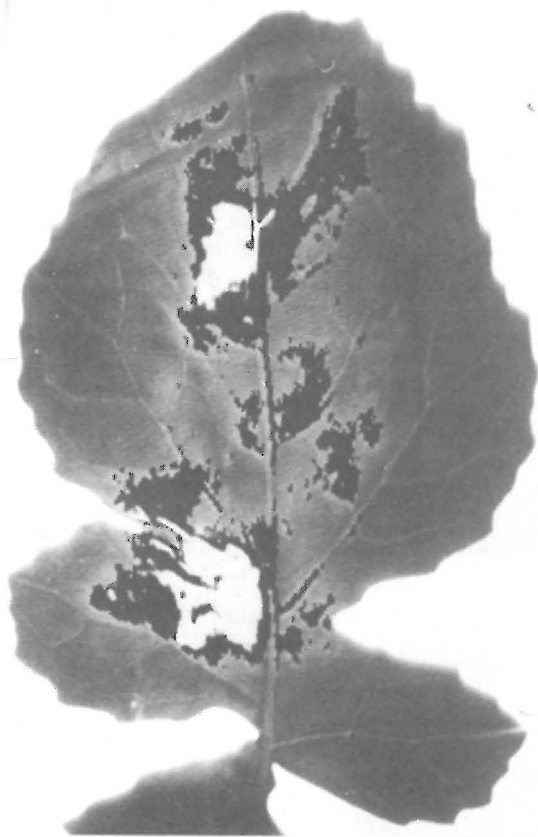


Fig. 7. A leaf of *Brassica napus* var. *oleifera* Metzger infected by *Verticillium dahliae* Kleb.

Copy of Kodachrome-II film of the leaf displayed on the colour-TV shown in Figure 2:f.

Total leaf area = 129912 pixels.
 Severe infection (centre, light grey area) = 4.36 %;
 moderate infection (dark grey area) = 8.85 %;
 surrounding area = 86.79 %.



Fig. 8. A wheat leaf (cultivar C = Vernal) infected by leaf rust, *Puccinia recondita* var. *tritici*. Copy of a Kodachrome-II film. Sorus area (black spots), surrounded by a green halo (dark grey area), and outside the spots a healthy area (light grey).

Table 2. Leitz-TAS automatic analysis of wheat leaves infected by leaf rust as shown in Figure 8.

Cultivar	Number of sori	Total leaf area (pixels)	Sorus area (%)	Green halo area (%)	Sorus + green halo area (%)	Sorus/ (green halo + sorus) area (%)	Mean size of	
							Sori (pixels)	Sori + green halo area (pixels)
a	8	11984	9.41	24.26	33.67	27.96	141.0	504.4
b	7	11998	3.18	8.89	12.07	26.31	54.4	206.8
c	23	15552	5.90	13.32	19.22	30.68	39.9	130.0

The instrument was not calibrated in mm or microns, and the areas are thus given in number of pixels.

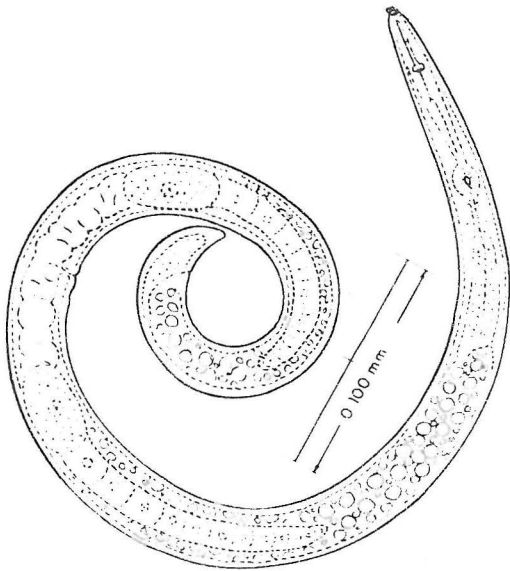


Fig. 9. *Helicotylenchus* sp. (female), a spiral nematode parasitic on many plants.

A copy of a drawing in *Introduction to research on plant nematology. An FAO guide to the study and control of plant-parasitic nematodes.* by Albert L. Taylor, 1967, p. 121 (FAO, Rome).

This and similar nematode species are difficult to measure by conventional methods. By image reading instrument systems, such as by Leitz-TAS, it is easy to get a quick and accurate "automatic" measurement of the nematode body and its various organs.

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