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USE OF STAINS FOR NEMATODE VIABILITY IN ASSESSING FUNGAL PARASITISM OF CYST NEMATODES

by

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Summary. Stains were used to distinguish live and dead *Heterodera avenae* eggs exposed to nematophagous fungi in cereal fields in eastern Scotland. Results indicated that although New Blue "R" could detect the dead eggs it could not be effectively substituted for egg plating techniques and that fluorescent stains should be used to differentiate eggs killed by fungi.

In a review on nematophagous fungi, Kerry (1984) stressed that a major difficulty in nematophagous fungi research was the lack of a quantitative technique for estimating the fungal propagules in soil and in parasitised nematodes. These difficulties can affect the assessment of the incidence of nematophagous fungi and their effects on nematodes. Different nematophagous fungi require different techniques depending upon their biology and mode of action against nematodes (Barron, 1982).

Several stains have been referred to in the literature as distinguishing live from dead nematodes (Shepherd, 1962; Moriarty, 1964; Chaudhuri *et al.*, 1966; Jatala, 1969; Bird, 1979). Most of these stains were used to distinguish dead cyst nematodes following the use of nematocidal chemicals or physical control methods, e.g. heat. Investigations were undertaken to assess their use for the detection of eggs of *Heterodera avenae* Woll. killed by nematophagous fungi in cereal fields in Eastern Scotland (Boag and Lopez, 1989).

Materials and methods

To extract the cysts, soil samples (500-700 g moist soil) were wet sieved through 1,000 μm and 250 μm sieves to break up the soil aggregates then processed using a fluidising column (Trudgill *et al.*, 1972). Hand-picked cysts were crushed, the eggs separated from the cyst debris by filtering through a 250 μm sieve and then collected on a Sintaglass filter No. 3 (Gallenkamp, Loughborough, England). New Blue "R" (Shepherd, 1962; Moriarty, 1964) and chrysoidin (Moriarty, 1964) were used to assess the presence of dead eggs of *H. avenae* using the following technique. Aqueous solutions of the stains of both chrysoidin (0.005%) and New Blue "R" (0.05%) had eggs added to them and kept at 20 °C. A preliminary test showed that the recommended times of staining, two days for chrysoidin and seven days for New Blue "R" were not sufficient, so batches of eggs were stained for up to seven days (chrysoidin) and 14 days for New Blue "R". The stain was then

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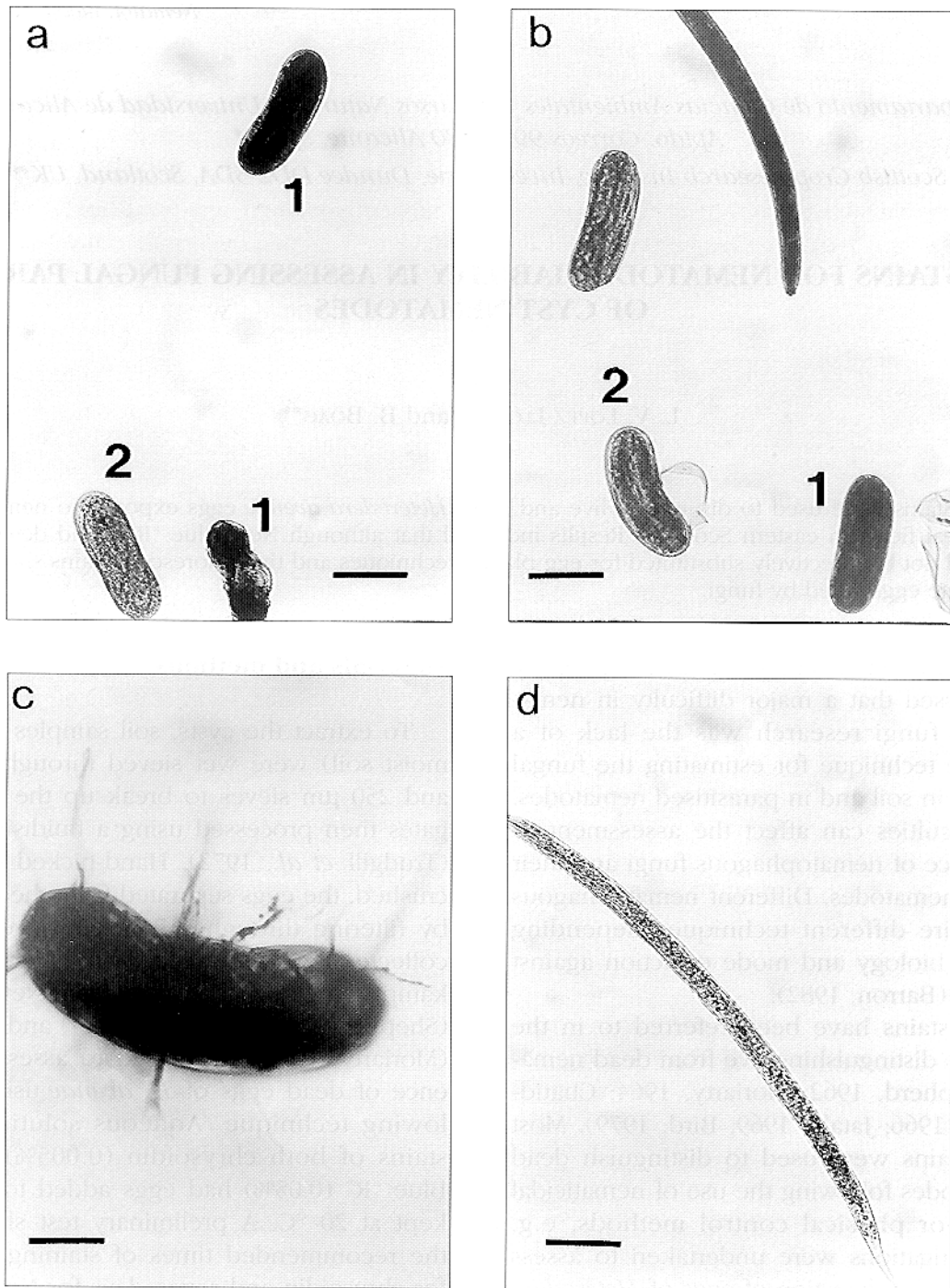


Fig. 1 - Staining of *Heterodera avenae* eggs with New Blue "R": a, (1) dead eggs deeply stained, (2) live egg slightly stained showing a juvenile inside; b, dead juvenile strongly stained, also dead (1) and live (2) eggs; c, fungal infected egg positively stained; d, live juvenile not stained. Bars: a, b, d = 50 μ m; c = 25 μ m.

washed from solution by diluting with distilled water until the water showed no stain and the eggs scored as stained or not under a dissecting microscope (x 45 magnification). Those eggs that were difficult to ascertain as stained or not were scored using x 90 magnification. Photographs were taken with a Zeiss microscope using Ilford PAN F 135 film.

Results and discussion

Virtually all *H. avenae* eggs which had been in chrysoidin were stained yellow and only occasionally was it possible to distinguish any orange eggs, even when magnifications of x 90 were used. With New Blue "R", two different types of staining were observed. Eggs with normal contents (most with a juvenile) were lightly stained [Fig. 1 a (2), b (2)], whereas eggs with distorted contents i.e. no juveniles, half empty, black, oily or with detectable fungal material showed intense staining i.e. deep purple to black [Fig. 1 a (1), b (1), c]. Juveniles that had already hatched prior to being put in the stains and were free in the egg suspensions exhibited two types of staining. Dead juveniles stained strongly, similar to dead eggs [plate 1 (b)], whereas live juveniles were very little stained (Fig. 1d).

Moriarty (1964) found that live eggs of *H. schachtii* Schm. stained diffuse yellow with orange granules with chrysoidin and did not

stain with New Blue "R". Shepherd (1962) immersed *Heterodera* eggs in New Blue "R" and found that live eggs did not take up the stain. In our study the light, pale blue staining of live eggs was probably due to overstaining *H. avenae* eggs, especially as there were no cysts in the suspension. The New Blue "R" did differentiate live from dead eggs, and fungal infected eggs stained as "dead eggs". With chrysoidin the differences between live and dead eggs were not obvious, but since occasionally whole eggs stained orange overstaining may have obscured the differences.

The percentages of *H. avenae* eggs positively stained with New Blue "R" in two samples from the soil survey by Boag and Lopez-Llorca (1989) are shown in Table I. These were compared with the percentage of eggs infected, as calculated by egg plating (Lopez-Llorca and Duncan, 1986), the numbers of resting spores of *Nematophthora gynophila* and *Verticillium chlamydosporium* (Crump and Kerry, 1981) and the numbers of eggs per gram of dried soil (Southey, 1970).

The higher percentages of eggs stained by New Blue "R" in sample 2 coincided with the higher percentage of eggs infected by fungi. The discrepancies between the percentages of the infected eggs and the percentage of dead eggs (estimated by staining with New Blue "R") may be explained by the fact that distorted eggs, namely eggs which were dead due to reasons other than fungal penetration, were also

TABLE I - Comparison of total percentage of eggs infected, numbers of resting spores g^{-1} soil of *Nematophthora gynophila* (N.g.), *Verticillium chlamydosporium* (V.c.), percentage of eggs positively stained by New Blue "R" and numbers of eggs g^{-1} soil of two samples (30, 31) of suppressive soils for *Heterodera avenae* from a survey in eastern Scotland.

Sample	Total % eggs infected	No. resting spores g^{-1}		% Eggs stained by New Blue "R"	<i>H. avenae</i> eggs g^{-1} soil
		N.g.	V.c.		
1	16.4±0.8	137.6	54.4	66.8±4.1	59.0±1.5
2	41.0±4.1	85.3	32.2	81.9±4.6	8.9±0.4

stained by new Blue "R". These distorted eggs were not scored as infected eggs.

Our results indicate that staining with New Blue "R" can detect dead eggs but cannot adequately be substitute for egg plating techniques. However, fluorescent stains e.g. fluorescein diacetate (Soderstrom, 1977) may in the future be used to differentiate eggs which have died due to fungal parasitism.

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