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Comparative Morphometrics of Eggs and Second-Stage Juveniles of *Heterodera schachtii* and a Race of *H. trifolii* Parasitic on Sugarbeet in The Netherlands[1]

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Abstract: Measurements of second-stage juveniles of *Heterodera schachtii* from California and The Netherlands and a race of *H. trifolii* from The Netherlands were obtained and compared to determine if these populations can be differentiated by morphometrics. Juvenile lengths of 10 specimens from each of 10 cysts of each population were measured. Dimensions of tail regions of 20 juveniles from individual cysts of *H. schachtii* (California) and a like number of juveniles of *H. trifolii* (The Netherlands) were also obtained. The mean lengths of juveniles of *H. schachtii* from California and The Netherlands were not significantly different, but similar measurements of *H. schachtii* and *H. trifolii* were different ($P = 0.05$). Mean dimensions of tail lengths, tail widths, tail hyaline lengths, and tail length/tail width were significantly greater for *H. trifolii* than for *H. schachtii*. Also, dimensions of eggs of *H. trifolii* were significantly greater than dimensions of *H. schachtii* eggs. The investigations established that *H. schachtii* can be readily differentiated from *H. trifolii* by morphometrics of eggs and juveniles. Minimum sample sizes required for specified confidence intervals for each criterion measured are provided.

Key words: measurements, differentiation, clover cyst nematode, sugarbeet nematode, *Beta vulgaris* L.

In 1975, a race of *Heterodera trifolii* Goffart, 1932 attacking sugarbeet, *Beta vulgaris* L., and other plant species within the families Chenopodiaceae, Cruciferae, Polygonaceae, Caryophyllaceae, and Leguminosae (7,16) in The Netherlands was described. This race of *H. trifolii* was termed the Yellow Beet Cyst Nematode (YBCN). The YBCN has not yet been reported in the United States. Because the YBCN and the sugarbeet nematode, *Heterodera schachtii* Schmidt, 1871, have similar host ranges, it is possible that both species may occur in mixed populations. Consequently, a reliable method is needed to differentiate these species. We examined the feasibility of differentiating YBCN and *H. schachtii* by dimensions of eggs and other easily-measured morphological features of second-stage juveniles.

Cysts of *Heterodera* spp., whether from field or container-grown plants, vary greatly in size (6). This variability is caused by factors such as age of females, high population densities of nematodes on host-plant roots, and development of nematodes at sub-optimal temperatures or on unsuitable hosts. If similar variability in size occurs among juveniles within cysts, identification of species based upon morphometrics of juveniles would be confounded. Consequently, we examined the effect of cyst size on length of juveniles recovered from them.

MATERIALS AND METHODS

Mature cysts containing second-stage juveniles (J2) and eggs of *H. schachtii* from Salinas, California, and The Netherlands and the YBCN race of *H. trifolii* from The Netherlands were recovered from roots of sugarbeets grown in sandy-loam soil in a greenhouse.

Experiment 1: Cysts from each of the three populations were separated into two groups of 10 large and 10 small cysts to compare the effect of cyst size on range, mean, and variability of lengths of J2. The largest and smallest cysts within each group were measured. Single cysts of each of the six groups were placed in 5 ml of tap water in individual hatching cups (14,15) and incubated at 24 C in the dark. Juveniles emerging from cysts were collected daily, immediately killed in hot 0.5% aqueous acetic acid, and fixed in formalin:ethanol (4:1) (13). The remaining cysts were broken open and the live J2 removed and preserved as above. Ten randomly selected J2 from each cyst were mounted in fixative on glass slides and their body lengths measured.

Experiment 2: Lengths and widths of tails

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TABLE 1. Maximum and minimum measurements in mm of the largest and smallest of 10 cysts within two size groups of *Heterodera schachtii* from California and The Netherlands and the sugarbeet race of *H. trifolii* YBCN from The Netherlands.*

Cyst type	Parameter	*H. schachtii*				*H. trifolii*	
		California		The Netherlands		The Netherlands	
		Min.	Max.	Min.	Max.	Min.	Max.
Large	Length	0.6200	0.8783	0.8525	0.9817	0.7750	1.1367
	Width	0.3875	0.5942	0.5167	0.5942	0.3875	0.6717
Small	Length	0.5167	0.7104	0.6200	0.7492	0.5683	1.1108
	Width	0.3358	0.4521	0.3358	0.4392	0.3100	0.6200

* Measurements of largest and smallest cysts within three populations each of 10 cysts.

and the lengths of the hyaline portions of J2 tails were measured. One J2 from each of 20 cysts of *H. trifolii* or the California population of *H. schachtii* were examined. Camera lucida drawings were made of tail regions of J2 selected from each of the three populations.

Experiment 3: Lengths and widths of 10 eggs removed from each of six cysts of *H. trifolii* and of the California population of *H. schachtii* were measured. Only eggs containing fully developed live J2 were selected.

For each study, morphometric data were analyzed for significant differences between means by analysis of variance. Calculations were made for each measured variable of minimum sample size required to assure finding significant differences between species sample means. We also estimated the number of cysts needed for the 95% confidence limits calculated for a sample mean to be within a preselected percentage of that mean. Morphometric data of the J2 tail regions were analyzed to determine if measurements of tail length, tail width, and hyaline-portion tail length were significantly different between *H. trifolii* and *H. schachtii* and whether measurements of these parameters were correlated among the nematode species. All measurements were made with a microscope equipped with an ocular micrometer.

RESULTS AND DISCUSSION

Experiment 1: Table 1 lists the maximum and minimum lengths and widths of *Heterodera* cysts selected for evaluation of morphometrics of contained juveniles. Analysis of J2 lengths of *H. trifolii* and both populations of *H. schachtii* indicated that the variance among small cysts was greater than for large cysts. Therefore, a separate analysis of variance was performed for J2 within both the small and large cyst groups. Measurements of cysts selected for each group are presented in Table 1. No significant difference was found between mean lengths of J2 populations of *H. schachtii* from California and The Netherlands ($P = 0.05$), except the J2 removed from California cysts were longer than those that were allowed to emerge from cysts (Table 2). No overlapping of measurements of *H. schachtii* and *H. trifolii* J2 occurred; mean lengths of *H. trifolii* juveniles were significantly greater than for juveniles of either population of *H. schachtii.*

Measurements of *H. schachtii* juveniles were comparable to those reported by Raski (12) (Table 2). The mean length of *H. trifolii* juveniles of our YBCN population was greater than reported by other workers for other populations (2,3,5,7,8,10). The maximum length of YBCN J2 was also greater than previously reported measurements of *H. trifolii* J2 (4,5,11,19). Even greater values of mean and range (585 μm and 490–675 μm, respectively) were found by A. M. Golden who examined 50 juveniles of our population of *H. trifolii* YBCN from The Netherlands (pers. comm.).

Variance in lengths of J2 from several cysts was significantly greater ($P = 0.05$) than the variance in lengths of J2 from the same cyst. Thus, standard error of a mean can be reduced more effectively by increasing the number of cysts included in a sample rather than by increasing numbers of juveniles examined per cyst. The relationship between the least significant difference (LSD 0.05) for comparing sample

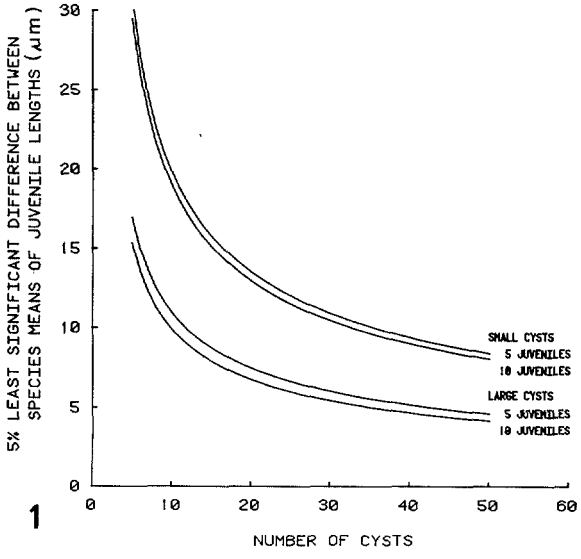
TABLE 2. Comparisons of lengths in micrometers of second-stage juveniles taken from large or small cysts* of two populations of *Heterodera schachtii* and the YBCN race of *H. trifolii*.

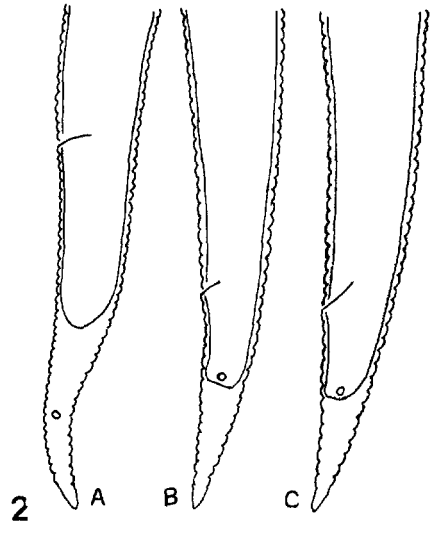
Statistic	*H. schachtii*			*H. trifolii*
	California		The Netherlands	The Netherlands
	Juveniles hatched from large cysts*			
Range	385.1–486.5		405.4–486.5	513.5–617.6
Mean†	432.7 b		442.0 b	578.5 a
SE of mean	3.600		3.483	3.159
	Juveniles removed from cysts	Juveniles hatched from small cysts*		
Range	389.2–513.5	405.4–473.0	378.4–479.7	493.2–628.4
Mean†	461.6 b	435.5 c	430.6 c	547.1 a
SE of mean	8.371	4.217	5.045	7.497

* Ranges of cyst sizes given in Table 1.

† Means of 100 juveniles (10 juveniles from each of 10 cysts). Means within a row not followed by the same letter differ significantly according to Duncan's multiple-range test ($P = 0.05$).

means and numbers of J2 examined in each cyst and number of cysts sampled is shown in Figure 1. An increase from 5 to 10 in the number of J2 examined per cyst does not greatly decrease the LSD 0.05, whereas increasing numbers of cysts by 10 or more results in a large decrease of the LSD 0.05, particularly with sample sizes of less than 20 cysts. Table 3 shows the number of cysts required to obtain a significant difference between mean lengths of *H. trifolii* and *H. schachtii* J2s (that is, one as large as the LSD 0.05 value) for specified + and − percentages of the *H. schachtii* sample mean. As would be expected, the sample size needs to increase exponentially as the desired





FIGS. 1, 2. 1) The number of large and small nematode cysts required to obtain least significant differences ($P = 0.05$) between mean lengths measurements of juveniles of *Heterodera schachtii* from The Netherlands or California and *H. trifolii* from The Netherlands with sample sizes of 5 and 10 juveniles measured per cyst are illustrated in a computer-generated graph. Units on vertical (Y) axis are given in μm. 2) Camera lucida drawings of the tail regions of second-stage juveniles of *Heterodera trifolii* parasitic on sugarbeet YBCN (A) and *H. schachtii* from The Netherlands (B) and California (C) (× 960).

TABLE 3. Number of cysts required for the mean of *Heterodera trifolii* data to be significantly larger than that of *H. schachtii* data if the true difference is a specified percentage of the sample mean for *H. schachtii.**

Variable†	Cyst type	Number of juveniles or eggs per cyst							
		5% difference		10% difference		15% difference		20% difference	
		5	10	5	10	5	10	5	10
Juvenile length	Large	8	6	3	3	3	3	3	3
	Small	22	21	7	6	4	4	3	3
Egg length	—	30	28	9	8	5	5	4	3
Egg width	—	18	15	6	5	4	3	3	3
Tail length‡	—	62		17		8		5	
Tail width‡	—	130		33		15		9	
Hyaline length‡	—	38		11		6		4	
c'‡§	—	71		19		9		6	

* Variance estimates from observations on both species. Type I and Type II error rates both assumed to equal 5%.

† Estimates based on 10 juveniles from each of 10 large and 10 small cysts for each species from The Netherlands and on 10 eggs from each of six cysts for each species from The Netherlands. Estimates for tail regions based on observations of one juvenile from each of 20 cysts for each species from The Netherlands.

‡ Estimates for these variables valid for one juvenile per cyst only.

§ Tail length/tail width.

level of detectable difference decreases. Table 4 gives the number of cysts required so that 95% confidence limits calculated for each sample mean will be within 1, 5, or 10% of that mean.

Experiment 2: The mean and maximum measurements of tail and hyaline lengths for the sugarbeet race of *H. trifolii* (Table 5) were greater than the measurements reported previously (4,7). Means of tail length, tail width, hyaline length, and c' (tail length/tail width) of the YBCN were significantly greater ($P = 0.05$) than corresponding means for *H. schachtii.* In addition, measurements of tail length, hyaline length, and c' ratios for the two *Heterodera* species did not overlap, indicating that any of these criteria should effectively differentiate the two nematode species. Also, tail lengths and tail hyaline lengths of J2s were highly correlated for both species (Table 6). The morphology of J2 tails of *H. trifolii* and the two *H. schachtii* populations are illustrated in Figure 2. Table 3 shows for each tail measurement the number of cysts required to obtain a difference between species sample means at least as great as the LSD 0.05 value if the

TABLE 4. Number of cysts required for estimated 95% confidence limits to be within a specified percentage of the mean measurement of eggs and second-stage juveniles with sample sizes of 5 and 10 juveniles or eggs per cyst.

Variable	*Heterodera* species	Cyst type	1% of mean		5% of mean		10% of mean	
			5 juveniles	10 juveniles	5 juveniles	10 juveniles	5 juveniles	10 juveniles
Juvenile length*	*H. trifolii*	Large	17	14	3	3	2	2
		Small	59	54	4	4	3	3
	H. schachtii	Large	28	23	3	3	2	2
		Small	94	86	6	5	3	3
Egg length†	*H. trifolii*	—	102	94	6	6	3	3
	H. schachtii	—	132	121	7	7	3	3
Egg width†	*H. trifolii*	—	64	52	4	4	3	3
	H. schachtii	—	77	63	5	4	3	3

* Estimates based on observations of 10 juveniles from each of 10 cysts of each size of *H. trifolii* and of *H. schachtii* from The Netherlands.

† Estimates based on observations of 10 eggs from each of six cysts of *H. trifolii* from The Netherlands and of *H. schachtii* from California.

TABLE 5. Morphometrics of selected characters in the tails of *Heterodera* spp. J2.*

Nematode species	Statistic	Tail length	Tail width	c'†	Hyaline‡ length
H. schachtii	Range	33.8–52.7	12.2–16.9	2.78–4.00	18.9–28.4
(California)	Mean§	45.5	13.4	3.4	24.4
	SE of mean	0.96	0.23	0.065	0.58
H. trifolii	Range	63.5–75.7	12.8–14.9	4.27–5.50	33.8–47.3
(YBCN Netherlands)	Mean§	69.9	14.1	5.0	37.9
	SE of mean	0.72	0.15	0.071	0.73

* All measurements given in micrometers.
† Tail length/tail width.
‡ Clear area in distal end of tail region.
§ Mean of 20 juveniles each from a separate cyst. Means for *H. trifolii* are significantly larger than those for *H. schachtii* for all measured variables ($P = 0.05$).

true difference were a specified percentage of the *H. schachtii* sample mean. Table 7 presents the estimated number of cysts needed for 95% confidence limits calculated for each sample mean to be within 1, 5, or 10% of that mean for each of the aforementioned tail measurements.

Experiment 3: As with morphometric data on juveniles, mean egg dimensions (length and width) were greater for the YBCN than reported by others (2,4,9) for races of *H. trifolii* parasitic on various legumes. Mean dimensions of eggs of *H. trifolii* were significantly greater ($P = 0.05$) than those of *H. schachtii* (Table 8), although the ranges overlapped. The relationship between the LSD 0.05 values comparing the two species for mean length or width of eggs and the number of cysts sampled are graphed in Figures 3 and 4. As with data on juveniles, the variance in egg dimensions among cysts was significantly greater ($P = 0.05$) than that within cysts; therefore, a larger decrease in the LSD 0.05 can be achieved by increasing numbers of cysts included in the sample than by increasing numbers of eggs examined per cyst. Estimates of sample size requirements to achieve specified confidence limits (Table 4) and LSD 0.05 values (Table 3) also reflect the importance of taking small samples from multiple cysts rather than large samples from a few cysts.

The Netherlands population of YBCN can be easily differentiated from the two populations of *H. schachtii* by dimensions of eggs and selected morphological characters of second-stage juveniles. The morphometrics of cysts, eggs, and J2, however, may not provide reliable data for separating these *Heterodera* species on hosts other than sugarbeet. Venduska (18) reported statistically significant differences in length of eggs of *H. schachtii* removed from cysts which developed on sugarbeet ($\bar{x}$ 105.9), rape (*Brassica napus* L.) ($\bar{x}$ 109.7), and charlock (*Sinapsis arvensis* L.) ($\bar{x}$ 99.6) and in egg widths among sugarbeet ($\bar{x}$ 49.3), charlock ($\bar{x}$ 45.7), and rape ($\bar{x}$ 44.8). Analysis of variance applied to morphometrics of two nematode species enabled estimation of minimum sample sizes required to achieve preselected confidence interval widths and

TABLE 6. Estimated correlation coefficients (with significance probability in parentheses).*

Variable	*Heterodera* species	Tail width	c'†	Hyaline length
Tail length	*H. trifolii*	0.088 (n.s.)	0.647 (0.002)	0.753 (0.001)
	H. schachtii	0.556 (0.011)	0.673 (0.002)	0.760 (0.001)
Tail width	*H. trifolii*		−0.701 (0.001)	−0.063 (n.s.)
	H. schachtii		−0.239 (n.s.)	0.120 (n.s.)
c'	*H. trifolii*			0.579 (0.008)
	H. schachtii			0.775 (0.001)

* n.s. indicates correlation coefficient is not significant ($P = 0.10$).
† Tail length/tail width.

TABLE 7. Number of cysts required for estimated 95% confidence limits to be within a specified percent of the mean for several measurements in tail regions of second-stage juveniles of *Heterodera trifolii* and *H. schachtii.**

Variable	*Heterodera* species	1% of mean	5% of mean	10% of mean
Tail	*H. trifolii*	116	6	3
length	*H. schachtii*	270	12	5
Tail	*H. trifolii*	150	8	3
width	*H. schachtii*	165	8	4
Hyaline	*H. trifolii*	240	11	4
length	*H. schachtii*	565	24	7
c'†	*H. trifolii*	145	7	3
	H. schachtii	315	14	5

* Estimates based on observations of one juvenile hatched from each of 20 cysts.
† Tail length/tail width.

TABLE 8. Measurements in μm of fully developed embryonated eggs of *Heterodera trifolii* and *H. schachtii.*

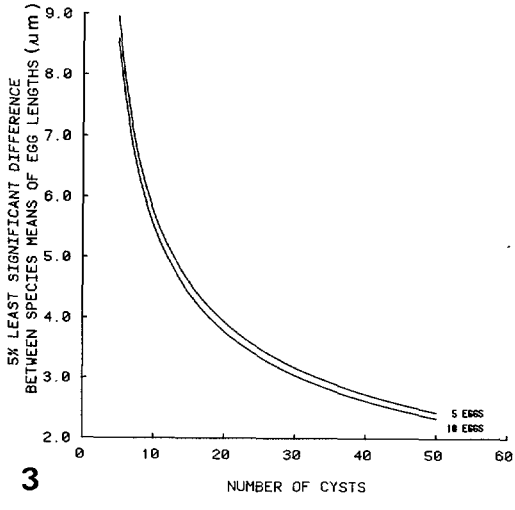
	H. trifolii, YBCN	*H. schachtii*, California
Egg length		
Range	105.1–135.6	84.8–122.0
Mean*	120.3	106.0
SE of mean	1.90	2.82
Egg width		
Range	42.4–59.3	37.3–50.9
Mean*	48.2	43.9
SE of mean	0.82	0.60

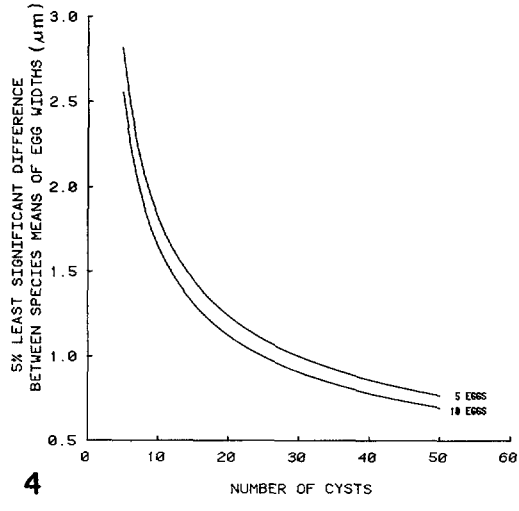
* Means of 60 eggs (10 eggs from each of six cysts). Means for *H. trifolii* are significantly larger than those for *H. schachtii* for both variables ($P = 0.05$).

LSD values. These results should be valuable to individuals concerned with the detection and identification of these sugarbeet parasites.

Early investigations suggest that *H. schachtii* and *H. trifolii* are closely related species. Nine bivalent chromosomes are formed at meiosis in *H. schachtii* which indicates the diploid number for this species is 18 (9). Mulvey (10,11) suggested that *H. trifolii* is a triploid (3n = 27) parthenogenetic form of *H. schachtii.* Chromosome numbers of 26, 27, and 28 have been reported for *H. trifolii* (17). Triantaphyllou and Hirschmann (17) discovered populations of *H. trifolii* having 33, 34, and 35 chromosomes and concluded that this nematode is a tetraploid parthenogenetic form that may have evolved from the closely related amphimictic species, *H. schachtii,* or perhaps from *H. glycines* (2n = 18). The soybean cyst nematode, however, is an unlikely ancestor, because it has not been found in Europe.

There is some evidence that suggests that YBCN may have evolved very recently from *H. schachtii* in Europe. The host range of the sugarbeet race of *H. trifolii* is remarkably similar to that of *H. schachtii* (7,16). Except for *H. cruciferae,* which does not





FIGS. 3, 4. The number of cysts required to obtain least significant differences ($P = 0.05$) between mean lengths (Fig. 3) or widths (Fig. 4) of eggs of *Heterodera schachtii* from California and *H. trifolii* from The Netherlands with 5 or 10 eggs measured per cyst.

parasitize sugarbeet, no other *Heterodera* species has a host range resembling that of *H. schachtii.* Dimensions of cysts, juveniles, and eggs of the YBCN are significantly greater than those of *H. schachtii.* On the other hand, mutations within a population of *H. trifolii* existing in The Netherlands may account for the apparently sudden appearance of the YBCN.

At the authors' request, Dr. A. C. Triantaphyllou examined 20 specimens of the YBCN race of *H. trifolii*; all had 35 and possibly 36 chromosomes and reproduced by mitotic parthenogenesis, indicating that YBCN is tetraploid. Among parthenogenetic species where differences in ploidy exist, there is a corresponding difference in size of individuals (4). In the study reported here, eggs and juveniles of the YBCN race *H. trifolii* were greater than those reported for other host races of this species.

Because all progeny of parthogenetic species have the potential of developing to egg-producing individuals, large populations can be generated in a relatively short time, thus enabling effective exploitation of suitable hosts (1). This exploitation in turn increases the likelihood of rapid dissemination if the spread is not limited by physical barriers or by the absence of host plants. The YBCN, discovered in 1975 (7), as yet remains confined to an area of light sandy soils in the southern part of The Netherlands, which suggests that it may have originated very recently in this area, and the likelihood of additional parthenogenetic polyploid taxa forming there within the *H. trifolii* complex is possible.

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