Journal of Nematology 27(2):206–212. 1995.

Morphometrics of Infective Juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida)[1]

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Abstract: Selected morphometrics of *Heterorhabditis bacteriophora* and seven species of *Steinernema* from in vivo culture were compared in relation to time of harvest. In addition, five *Steinernema* species were reared in vitro and their morphometrics were compared with those from in vivo culture. With in vivo culture, there was generally a negative linear relationship between body length of infective juveniles (IJ) and time of harvest. The distance from the anterior end to the excretory pore (EP) and the tail length (T) of IJ also varied with time of harvest. The E percentage (= EP/T × 100) was the least variable. Body lengths of IJ reared in vitro were much less than those of IJ reared in vivo. The study suggests that IJ harvested from in vivo culture within 1 week of emergence from cadavers are best for species identification. Infective juveniles from in vitro culture should not be used for species identification.

Key words: Culture, *Heterorhabditis bacteriophora,* entomopathogenic nematode, morphology, nematode, *Steinernema,* taxonomy.

Morphometrics of the infective juvenile (IJ) of Steinernematidae are important for description and identification of species. We have noticed that measurements of the IJ vary considerably depending upon the time of harvest after they first appeared in in vivo culture, and whether the nematodes were reared in vivo or in vitro. This study reports on morphometric variation of IJ harvested at 3-day intervals for six consecutive harvests from in vivo culture and compared to a single harvest from in vitro culture.

MATERIALS AND METHODS

In vivo rearing: The nematodes used in the experiment were *Steinernema anomali* (Kozodoi, 1984) Poinar 1990; *S. carpocapsae* (Weiser, 1955) Poinar, 1990 (strain Agriotos); *S. feltiae* (Filipjev, 1934) Poinar, 1990; *S. glaseri* (Steiner, 1929) Wouts et al., 1982; *S. intermedia* (Poinar, 1985) Poinar, 1990; *S. riobravis* Cabanillas et al., 1994; *S. scapterisci* Nguyen & Smart, 1990 (strain Uruguayan); and *Heterorhabditis bacteriophora* Poinar, 1975. All of the nematodes were reared in larvae of the greater wax moth *Galleria mellonella* (L), except for *S. scapterisci,* which was reared in the mole cricket, *Scapteriscus vicinus* (Scudder). For all nematodes except *S. scapterisci,* 10 final instar larvae of *G. mellonella* were exposed to 10,000 IJ in petri dishes (100 × 15 mm) lined with two moistened filter papers. After the insects died (about 3 days after exposure), they were placed on White traps (5) to harvest IJ. The same procedure was followed for *S. scapterisci* except for the use of *S. vicinus.* Infective juveniles were harvested 1 day after they first appeared in the White traps, and again at 3, 6, 9, 12, and 15 days after the first harvest.

In vitro rearing: Five species, *S. anomali, S. carpocapsae, S. feltiae, S. riobravis,* and *S. scapterisci,* were reared in vitro. To begin the rearing process, symbiotic bacteria were obtained from the nematodes by macerating surface–sterilized IJ in a tissue homogenizer (1). The macerate was streaked on nutrient agar. When colonies developed, bacteria from young colonies were transferred to test tubes containing 20 ml of brain–heart infusion. The test tubes were attached to a continuously operating shaker. After 24–48 hours the infusion became milky, indicating good growth of the bacteria.

Nematode eggs were collected from gravid females by placing them in a digesting–sterilizing solution (0.2 M sodium hypochlorite and 0.4 M sodium hydroxide); the solution was stirred frequently (3). After 10 minutes the solution digested the

Received for publication 12 September 1994.

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TABLE 1. Morphometrics of infective juveniles (IJ) of *Steinernema* spp. and *Heterorhabditis bacteriophora* from six harvests of in vivo cultures, 3 days apart, and from one harvest of in vitro culture of the first five *Steinernema* spp. (n = 20).

	Average measurements (μm) of IJ for each harvest						
	Reared in vivo						Reared in vitro
Character†	0	3d	6d	9d	12d	15d	
				Steinernema anomali			
L	1,227 (16) (1,018–1,341)	1,167 (16) (1,024–1,311)	1,232 (9) (1,140–1,287)	1,202 (12) (1,085–1,287)	1,128 (15) (994–1,207)	1,035 (16) (915–1,183)	993 (19) (766–1,188)
EP	96 (1) (84–103)	91 (1) (78–101)	98 (1) (84–108)	92 (2) (72–108)	92 (1) (80–108)	87 (2) (66–103)	85 (2) (70–98)
T	78 (1) (69–86)	82 (1) (75–92)	80 (1) (69–91)	80 (1) (72–91)	79 (1) (80–90)	76 (1) (63–84)	74 (2) (61–86)
E%	124 (2) (104–138)	112 (2) (100–122)	124 (2) (104–141)	115 (3) (87–133)	117 (3) (102–138)	115 (10) (98–138)	116 (3) (75–133)
				Steinernema carpocapsae			
L	562 (6) (524–616)	546 (7) (500–591)	520 (10) (445–604)	523 (13) (421–604)	533 (8) (470–598)	503 (9) (427–567)	531 (9) (463–579)
EP	38 (1) (34–45)	38 (1) (34–44)	35 (1) (30–39)	37 (1) (33–39)	37 (1) (34–38)	35 (1) (28–42)	37 (1) (31–44)
T	55 (1) (48–69)	53 (1) (48–59)	50 (1) (45–59)	49 (1) (39–61)	49 (1) (44–56)	45 (1) (36–50)	49 (1) (39–55)
E%	68 (2) (55–81)	72 (1) (63–88)	70 (1) (61–79)	75 (2) (64–89)	75 (1) (67–86)	77 (2) (61–92)	76 (2) (63–92)
				Steinernema feltiae			
L	914 (10) (841–994)	870 (10) (799–957)	854 (13) (701–963)	841 (12) (732–951)	785 (16) (671–896)	794 (6) (744–841)	632 (13) (524–707)
EP	61 (1) (50–69)	60 (1) (47–77)	61 (1) (56–77)	60 (2) (44–75)	55 (1) (44–64)	54 (1) (50–61)	51 (1) (43–59)
T	86 (1) (75–94)	84 (1) (72–94)	80 (2) (69–92)	77 (1) (58–88)	75 (2) (66–91)	79 (1) (73–86)	66 (1) (56–78)
E%	71 (5) (60–80)	71 (2) (58–98)	76 (2) (62–89)	78 (2) (58–94)	74 (1) (64–83)	68 (1) (60–74)	77 (1) (68–87)
				Steinernema riobravis			
L	635 (12) (561–738)	614 (9) (530–671)	616 (12) (537–701)	598 (10) (518–671)	570 (11) (457–665)	579 (12) (493–695)	457 (10) (365–567)
EP	55 (1) (50–64)	55 (1) (45–63)	55 (1) (48–61)	54 (5) (45–61)	47 (2) (34–59)	52 (3) (36–70)	38 (1) (31–45)
T	53 (1) (47–61)	51 (1) (42–56)	50 (1) (42–61)	50 (1) (41–56)	50 (1) (44–56)	50 (1) (39–56)	44 (1) (36–53)
E%	104 (2) (91–120)	108 (3) (85–137)	109 (2) (94–137)	108 (10) (88–123)	95 (4) (64–123)	106 (6) (68–125)	85 (2) (76–112)
				Steinernema scapterisci			
L	591 (6) (543–640)	543 (8) (488–585)	537 (4) (506–573)	532 (6) (451–591)	512 (11) (409–585)	499 (7) (432–554)	482 (6) (366–567)
EP	40 (1) (38–44)	39 (1) (34–44)	39 (1) (36–41)	39 (1) (34–44)	37 (1) (30–41)	37 (1) (31–42)	39 (1) (31–47)
T	54 (1) (47–59)	56 (1) (47–64)	51 (1) (47–56)	53 (1) (45–63)	48 (1) (42–52)	48 (1) (39–56)	47 (1) (39–55)
E%	74 (1) (67–80)	70 (1) (61–90)	76 (1) (67–87)	74 (2) (65–81)	76 (1) (67–85)	78 (2) (65–93)	83 (2) (67–96)
				Steinernema glaseri			
L	1,241 (24) (981–1,433)	1,464 (22) (1,256–1,610)	1,433 (21) (1,256–1,634)	1,302 (25) (1,079–1,439)	1,257 (24) (1,055–1,421)	1,306 (38) (726–1,530)	—
EP	104 (2) (91–122)	117 (1) (106–133)	114 (2) (98–123)	110 (2) (91–123)	110 (2) (89–119)	112 (2) (92–127)	—

TABLE 1. *Continued*

Character†	Average measurements (μm) of IJ for each harvest						
	Reared in vivo						Reared in vitro
	0	3d	6d	9d	12d	15d	
T	78 (2) (66–106)	82 (1) (67–92)	80 (1) (70–89)	81 (2) (69–102)	82 (2) (70–111)	81 (2) (56–97)	–
E%	135 (4) (98–156)	143 (3) (124–167)	143 (2) (125–160)	138 (3) (109–161)	136 (4) (80–163)	140 (5) (120–217)	–
			Steinernema intermedia				
L	803 (6) (762–866)	759 (7) (695–823)	709 (11) (628–780)	728 (11) (598–804)	726 (11) (634–793)	676 (9) (604–780)	–
EP	71 (1) (61–78)	67 (1) (61–73)	63 (1) (52–72)	66 (1) (61–80)	66 (1) (58–69)	61 (1) (53–69)	–
T	67 (1) (61–78)	69 (1) (59–75)	63 (2) (50–76)	66 (2) (55–78)	65 (1) (56–73)	62 (1) (53–69)	–
E%	107 (2) (85–121)	98 (2) (89–124)	102 (3) (84–138)	101 (3) (84–132)	102 (3) (79–109)	99 (2) (85–109)	–
			Heterorhabditis bacteriophora				
L	587 (4) (561–634)	605 (4) (579–634)	600 (5) (555–634)	572 (7) (518–640)	578 (5) (524–622)	565 (5) (524–604)	–
EP	107 (1) (92–113)	106 (2) (88–116)	108 (2) (84–114)	104 (2) (83–119)	104 (1) (94–113)	105 (1) (94–113)	–
T	91 (2) (83–111)	96 (1) (89–105)	92 (3) (55–120)	90 (2) (63–100)	92 (2) (78–113)	97 (1) (89–109)	–
E%	119 (3) (83–134)	111 (2) (89–125)	119 (4) (95–180)	117 (2) (95–153)	114 (3) (83–140)	108 (2) (86–121)	–

The first number in each column is the mean of 20 measurements. The number in parentheses immediately following the mean is the standard error. The two numbers in parentheses immediately below these numbers are the range.

†L = length; EP = distance from anterior end to excretory pore; T = tail length; E% = EP/T × 100; - = not reared in vitro.

female tissues and released the eggs. The eggs were rinsed three times with sterilized saline solution (NaCl 7.5 g, KCl 0.35 g, $CaCl_2$ 0.21 g, 100 ml water), collected by pipet, and transferred to rearing medium (17 g dehydrated brain–heart infusion, 0.01 g cholesterol, 0.5 ml corn oil, 8 g agar, 500 ml deionized water) in petri dishes (100 × 15 mm) (2). The dishes were sealed with Parafilm (American National Can, Greenwich, CT) and incubated at 25 C. After the eggs hatched, 10 drops of the prepared bacterial culture were added to the dishes. Infective juveniles were harvested 3 to 4 weeks after eggs were transferred to rearing medium. The 3–4 week harvest period is the normal time for harvesting IJ cultured in vitro.

All IJ from in vivo and in vitro culture were stored at 8 C for a week before they were measured. Live IJ then were mounted in water on glass slides with coverglass supports, sealed with Zut, and measured about an hour later when they became immobile. Twenty IJ per harvest were measured for a total of 1,060. The body length (L), the distance from anterior end to excretory pore (EP), and the tail length (T) were measured; the E% (= EP/T × 100) was calculated. Means, standard errors, ranges, and regression analyses were obtained by SAS (4).

RESULTS

In vivo rearing: Measurements of IJ varied among species and time of harvest (Table 1). For most of the species, the body was longest at the first harvest and generally became shorter at each harvest thereafter. *Steinernema glaseri* and *H. bacteriophora* IJ were shorter at the first harvest than at the second harvest. Body length and time of harvest were negatively corre-

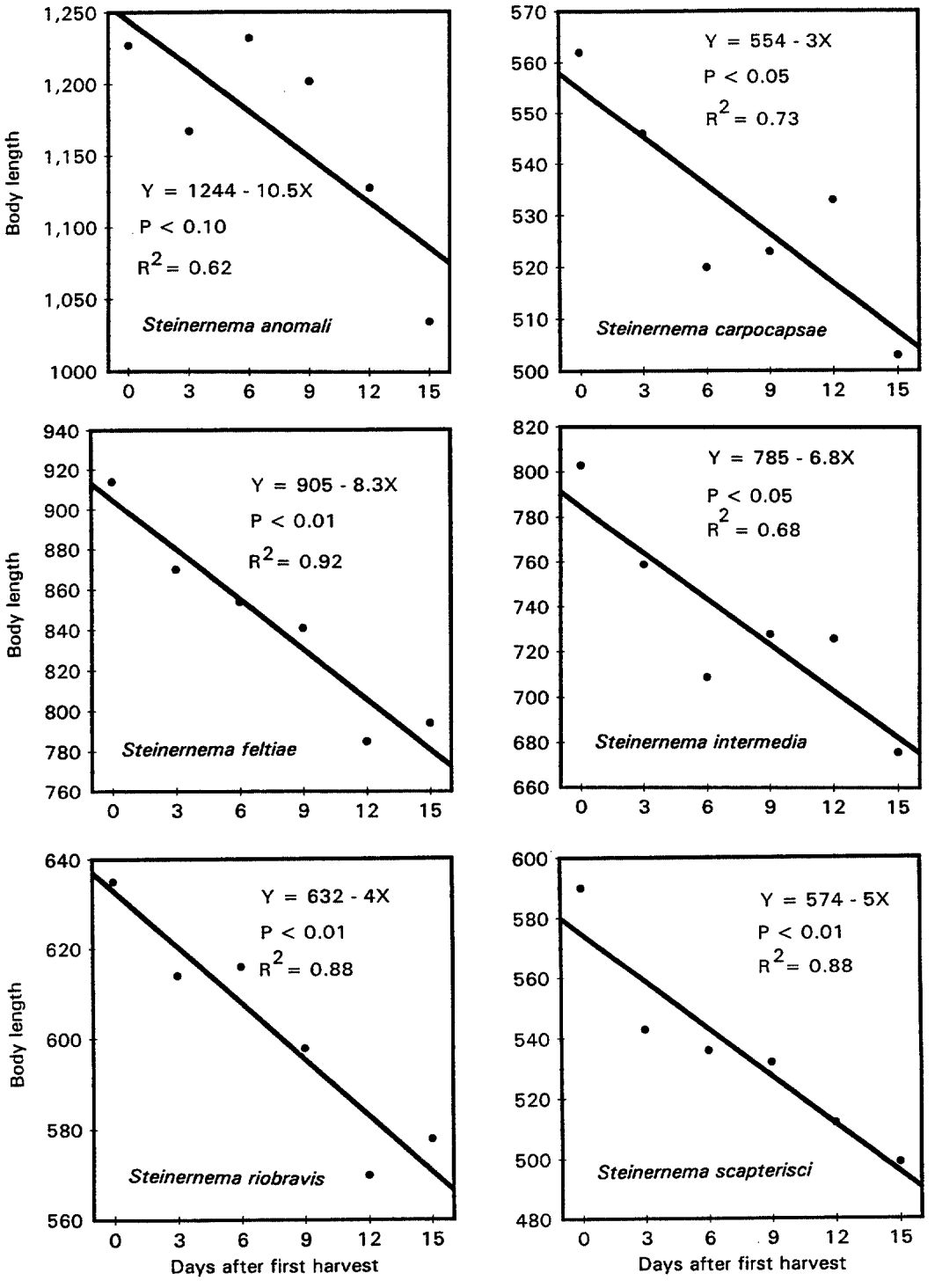

FIG. 1. Relationship between body length of infective juveniles and number of days after harvest of six species of *Steinernema*.

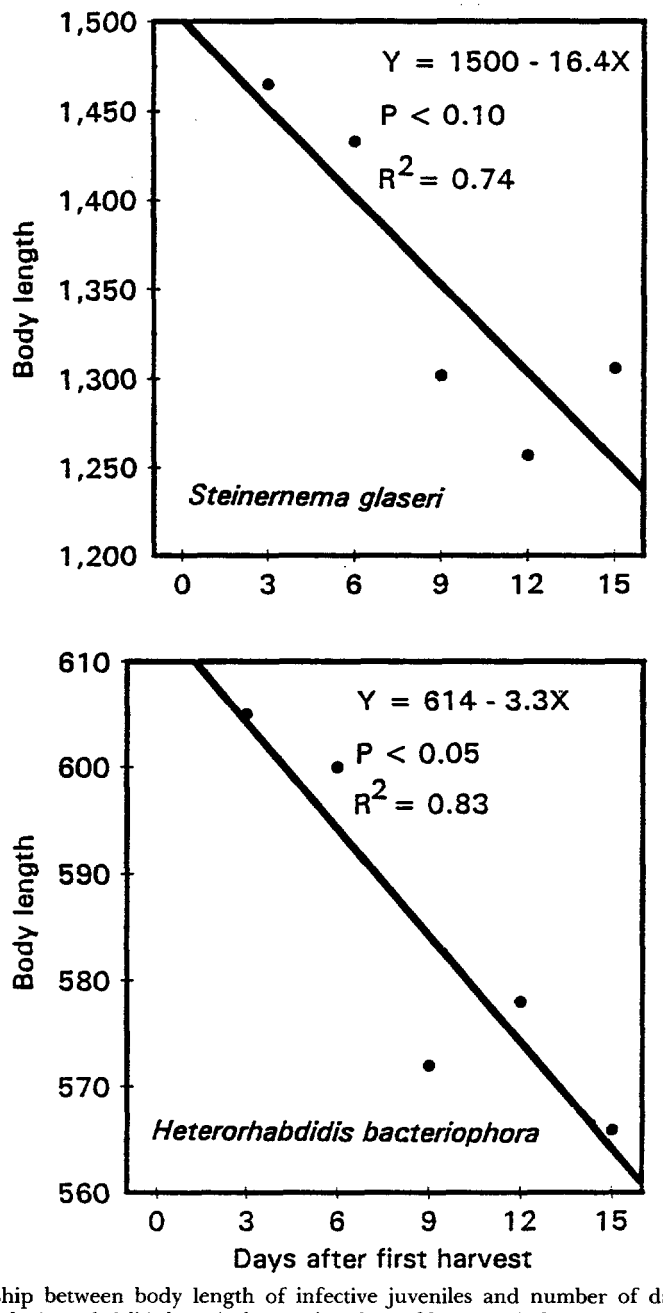

FIG. 2. Relationship between body length of infective juveniles and number of days after harvest of *Steinernema glaseri* and *Heterorhabditis bacteriophora,* using data of harvests 2–6.

lated except for *S. glaseri* and *H. bacteriophora* (Fig. 1). However, the relationship of body length and time of harvest for *S. glaseri* and *H. bacteriophora* was significant when the data for the first harvest were omitted from the data set before analysis (Fig. 2).

In general, there were fewer differences between harvests in measurements of EP and T than L (Table 1). Thus, a negative linear relationship between EP and time of harvest occurred only for *S. feltiae, S. intermedia,* and *S. scapterisci* (Fig. 3). A negative linear relationship between T and time of

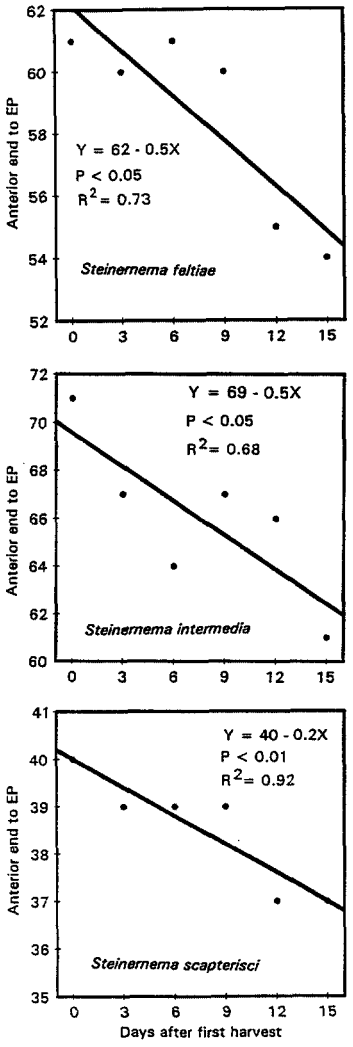

FIG. 3. Relationship between distance from anterior end to excretory pore (EP) and number of days after harvest of three species of *Steinernema.*

harvest occurred only for *S. carpocapsae, S. riobravis,* and *S. scapterisci* (Fig. 4). The linear relationship between the E% and the time of harvest was significant only for *S. carpocapsae,* making E% the least variable of the values in Table 1 (Fig. 5). This relationship was a positive correlation. In *S. glaseri,* T and E% were almost constant (Table 1).

In vitro rearing: The body length of IJ of all five species reared in vitro was less than that of first–harvest, in vivo–reared IJ, and, except for *S. carpocapsae,* was less than that of in vivo–reared IJ from the first five

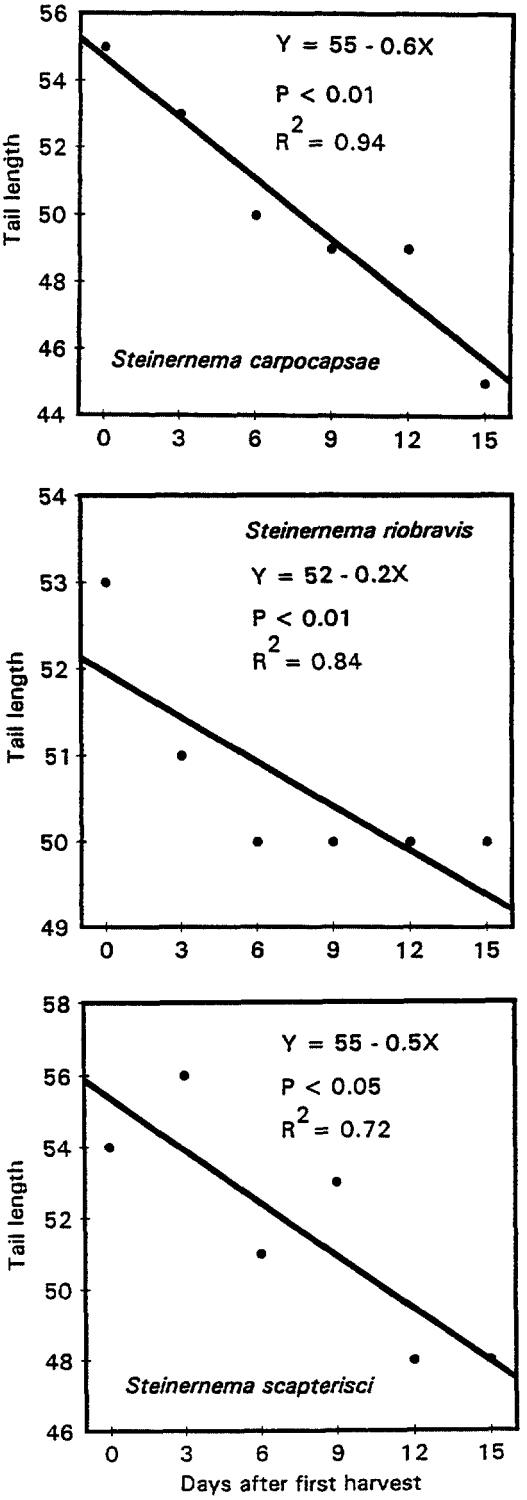

FIG. 4. Relationship between tail length and number of days after harvest of three species of *Steinernema.*

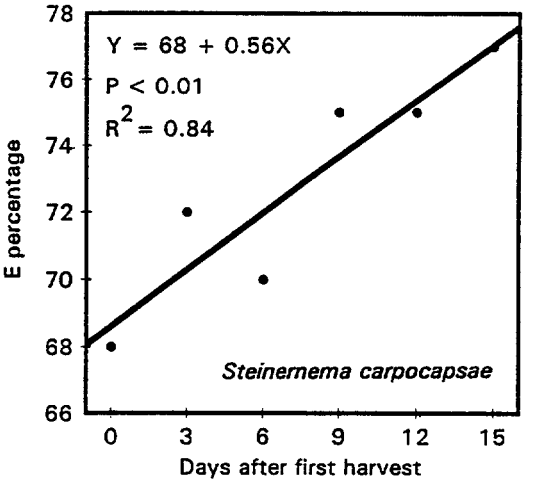

FIG. 5. Relationship between E percentage (= distance from anterior end to excretory pore divided by tail length × 100) and number of days after harvest of *Steinernema carpocapsae*.

harvests (Table 1). The EP, T, and E% were similar for in vivo– and in vitro–reared IJ except for EP in *S. riobravis,* T in *S. feltiae* and *S. riobravis,* and E in *S. riobravis* and *S. scapterisci* (Table 1).

DISCUSSION

Morphometrics of *Steinernema* spp. IJ from in vivo culture, especially the body length, vary significantly with time of harvest. To standardize future morphometric data for descriptions and identification of species, we suggest the use of IJ collected from in vivo culture over a 1-week period after their first emergence from the cadaver. It is probable that the size reduction of IJ over time from in vivo culture is related to a diminishing nutrient supply in the insect cadaver.

The E% value for each species differed little among harvests (Table 1). Along with morphological characteristics of other stages of *Steinernema* spp., the E% value may be useful for identification of species regardless of culture method or IJ harvest time.

All *Steinernema* species descriptions have been based on in vivo-reared nematodes. Since morphometrics, especially body length, of most IJ reared in vitro differ from those reared in vivo (Table 1), the use of morphometrics from in vitro-reared nematodes is not likely to fit the original descriptions. For example, body length means from in vivo–versus in vitro–reared IJ were: for *S. anomali,* 1,165 μm vs. 993 μm; for *S. feltiae,* 843 μm vs. 632 μm; for *S. riobravis,* 602 μm vs. 457 μm; for *S. scapterisci,* 536 μm vs. 482 μm (Table 1). Therefore, we suggest that morphometrics of IJ from in vitro culture not be used for taxonomic or identification purposes. However, morphometrics of *S. carpocapsae* strain Agriotos IJ reared in vivo and in vitro were similar. More studies on different strains of this species are needed to determine if rearing method affects morphometrics. When we took IJ from in vitro culture and reared them in an appropriate host, either *G. mellonella* or *Scapteriscus* spp., the resulting IJ had the morphometrics of IJ reared in vivo (unpubl. data). It would appear that essential elements for growth are lacking in culture media.

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