Influence of Incubation Solution on the Rate of Recovery of *Pratylenchus brachyurus* from Cotton Roots[1]

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Abstract: The rate of recovery of *Pratylenchus brachyurus* from cotton roots was enhanced when the tissue was incubated in solutions containing 10 ppm ethoxyethyl mercuric chloride, 50 ppm dihydrostreptomycin sulfate, 50, 100, or 1,000 ppm diisobutylphenoxethyl dimethyl benzyl ammonium chloride, or mixtures of these compounds. Incubation in 10 or 100 ppm zinc sulfate, zinc chloride, or magnesium chloride also enhanced the rate of recovery. Incubation solutions containing 1 or 1,000 ppm zinc chloride or magnesium chloride had no influence on this phenomenon, whereas, 10,000 ppm zinc sulfate, zinc chloride, or magnesium chloride retarded the rate of recovery. At all incubation intervals during the first 21 days after the roots were removed from soil, the *P. brachyurus* population consisted of approximately 25% second-stage juveniles, 44% third and fourth-stage juveniles, and 31% females. At least 88% of the second-stage juveniles and 51% of the third and fourth-stage juveniles passed through a single 325-mesh sieve, whereas, 84% of the females collected were retained on a sieve of this mesh. *Key Words: Gossypium hirsutum*, Extraction, Incubation.

A number of incubation techniques which can be used for the extraction of endoparasitic nematodes from root tissue have been described (1, 2, 5, 7, 8, 9, 11). In some cases non-nematicidal concentrations of fungicides and antibiotics have been added to incubation solutions to stimulate egg hatch or inhibit the growth and development of microbial contaminants (3, 6, 10). For several years the author has been extracting nematodes from roots by incubating the tissue in a mixture of ethoxyethyl mercuric chloride and dihydrostreptomycin sulfate in flasks on a gyratory shaker. It was recently discovered, however, that the incubation solution containing the fungicide and antibiotic was essential for nematode recovery equal or superior to that of other techniques. The present study, therefore, was undertaken to investigate the influence of various incubation solutions on the rate of recovery of *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, from cotton (*Gossypium hirsutum*) roots.

Materials and Methods

Greenhouse cultures of *P. brachyurus* maintained for 9–15 months in sandy loam soil planted with cotton ('Coker 201') were used as the source of nematode infested roots for ten incubation experiments (Table 1). Each incubation experiment was repeated at least once. The feeder roots were washed, cut into 1–2 cm segments, and mixed thoroughly. Root samples of 1, 2, or 5 g, depending on the experiment, were selected at random and placed in 50 or 250 ml flasks. The incubation solution under test (See Table 1 for complete listing of all compounds and mixtures tested) was added to each flask (15 ml to 50 ml flasks and 75 ml to 250 ml flasks), and the vessels were incubated on a gyratory shaker operating at 100 rpm. Distilled water was used, and each treatment was replicated 6–12 times. After

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TABLE 1. Solutions, concentrations, and time intervals used to evaluate the influence of incubation solutions on the rate of recovery of *Pratylenchus brachyurus* from cotton roots.

Experiment	Incubation solutions	Respective concentration (s) (ppm)	Incubation periods (days)
1	EMC[1]-DSS[2], H_2O	10–50	1,2,5,7,14,18,19
2	EMC-DSS, EMC, DSS H_2O	10–50, 10, 50	1,2,4,8,10
3	EMC-DSS, DPBA[3]-EMC, DPBA-DSS, DPBA, H_2O	10–50, 50–10, 50–50, 50	1,2,4,8,10
4	DPBA, H_2O	10, 100, 1,000, 10,000	1,2,4,8,10,14
5	$ZnCl_2$, $MgCl_2$, $HgCl_2$, H_2O	100	3,7,8,12
6	EMC-DSS, $ZnSO_4$, $ZnCl_2$, $MgCl_2$, H_2O	10	3,8,12
7	$ZnSO_4$, H_2O	1, 10, 100, 1,000, 10,000	1,2,4,8,10,12
8	$MgCl_2$, H_2O	1, 10, 100, 1,000, 10,000	1,2,4,8,10
9	$ZnCl_2$, H_2O	1, 10, 100, 1,000, 10,000	1,2,4,8,10
10	$HgCl_2$, H_2O	1, 10, 100	1,2,4,8,10

[1] Ethoxyethyl mercuric chloride (EMC)
[2] Dihydrostreptomycin sulfate (DSS)
[3] Diisobutylphenoxyethyl dimethyl benzyl ammonium chloride (DPBA)

pre-determined incubation periods (Table 1), each flask was removed from the shaker and the incubation solution poured through a 325, 400, and 500-mesh sieve, in succession. The nematodes collected on the sieves were rinsed with distilled water and suspended in 20 ml of water. Freshly prepared incubation solutions were immediately added to the root segments in the flasks and they were returned to the shaker for further incubation.

Recoveries of *P. brachyurus* in Experiments 2, 3, and 10 (Table 1) were determined by counting all of the nematodes in each sample, whereas, those in Experiments 1, 4, 5, 6, 7, 8, and 9 (Table 1) were determined by counting the nematodes present in three 1-ml aliquots and estimating the total population density.

The nematodes recovered during the first eight days of Experiments 4 and 6 (Table 1) were used to study the influence of incubation solutions on the viability of *P. brachyurus*. Aqueous suspensions of approximately 100 nematodes from one incubation treatment were added to methyl bromide-treated sandy loam in 10.2-cm plastic pots planted with 21-day-old cotton seedlings. Six replicate pots received nematodes recovered from each incubation solution. After 60 days in a greenhouse, the potted soil and cotton plants were transplanted into 35-cm plastic pots. Three months after transplanting, 10 g of roots and 100 g of soil were taken at random from each pot, and analyzed for the presence of *P. brachyurus*.

In an additional test, cotton roots containing *P. brachyurus* were incubated in 100 ppm zinc sulfate for 21 days. Recovery densities were determined after 1, 2, 7, 14, and 21 days, and the nematode-containing residues from the three sieves were collected separately. Each specimen recovered was classified as either an adult, third-fourth-stage juvenile, or second-stage juvenile.

RESULTS

Incubation of cotton roots in a solution containing 10 ppm ethoxyethyl mercuric chloride (EMC) and 50 ppm dihydrostrep-

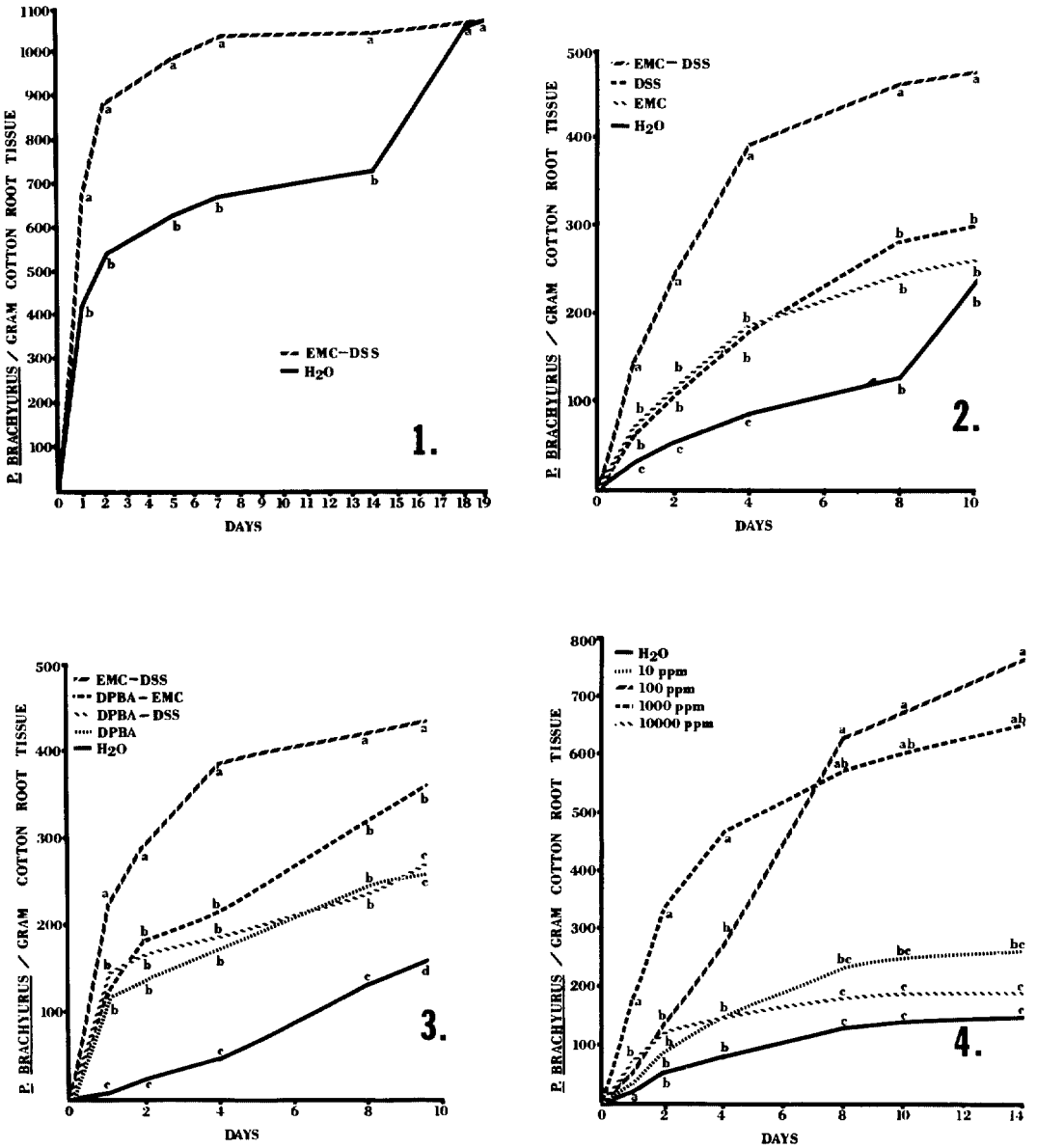


FIG. 1–4. Recovery of *Pratylenchus brachyurus* from cotton roots incubated in various concentrations of incubation solutions. Population densities on a given day marked with same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range test. **1.** Ten ppm ethoxyethyl mercuric chloride (EMC) and 50 ppm dihydrostreptomycin sulfate (DSS). **2.** Ten ppm ethoxyethyl mercuric chloride, 50 ppm dihydrostreptomycin sulfate (EMC), or a mixture of these compounds. **3.** Ten ppm ethoxyethyl mercuric chloride (EMC), 50 ppm diisobutylphenoxyethyl dimethyl benzyl ammonium chloride (DPBA), 50 ppm dihydrostreptomycin sulfate, or various mixtures of these compounds. **4.** Four concentrations of diisobutylphenoxyethyl dimethyl benzyl ammonium chloride (DPBA).

tomycin sulfate (DSS) resulted in a more rapid recovery of *P. brachyurus* than in water-incubated controls (Fig. 1). The difference between recovery rates was evident after the initial 24-hr incubation period, and remained significantly different for two weeks. The final cumulative recoveries, however, were equivalent. Approximately 90% of the *P. brachyurus* were recovered after 48 hr in EMC-DSS, whereas, this level of recovery was not attained with water until the roots had been incubated for approximately 16 days.

The rate of recovery of *P. brachyurus* from cotton roots incubated in 10 ppm EMC or 50 ppm DSS was significantly less than in an EMC-DSS mixture (Fig. 2). Recovery from this mixture was equivalent to the additive effects of the components. While the individual components of the mixture initially stimulated nematode recovery, the accumulative recovery after 8 days was not significantly different from that in water. An incubation solution of 50 ppm of diisobutylphenoxethyl dimethyl benzyl ammonium chloride (DPBA), used alone or in combination with 10 ppm EMS or 50 ppm DSS, enchanced the rate of recovery of *P. brachyurus* from cotton roots (Fig. 3); it was, however, not as rapid as in the 10–50 ppm EMS-DSS mixture incubation solution. The rate of recovery was influenced by the concentration of the incubation solution (Fig. 4). Both 100 and 1,000 ppm of DPBA enhanced the rate of recovery, whereas, the results with 10 and 10,000 ppm were not significantly different from that of water.

Use of incubation solutions containing 100 ppm zinc chloride or magnesium chloride, resulted in a similar response, and significantly increased the rate of recovery of *P. brachyurus*, whereas, 100 ppm mercuric chloride resulted in a significantly reduced cumulative recovery (Fig. 5). At 10 ppm, neither zinc chloride nor magnesium chloride enhanced the recovery rate (Fig. 6). In general, the responses to incubation solutions of zinc sulfate, zinc chloride, and magnesium chloride were similar (Figs. 7, 8, 9). One hundred ppm of these salts greatly enhanced the rate of recovery, whereas 10 ppm gave a limited response. Except in the case of 1,000 ppm zinc sulfate, 1,000 ppm and 1 ppm zinc sulfate, zinc chloride, and magnesium chloride had no significant influence on the rate of recovery. Concentrations of 10,000 ppm appeared to retard the process. Incubation in a solution of mercuric chloride influenced the rate of recovery in a different manner. A concentration of 1 ppm had no influence, whereas, 10 ppm initially stimulated recovery (Fig. 10). Stimulation with 10 ppm was significant for only 8 days, whereas, incubation in 100 ppm was inhibitory.

After incubating cotton roots in 100 ppm zinc sulfate for five intervals over a period of 21 days, the final cumulative *P. brachyurus* recovery consisted of 25% second-stage juveniles, 44% third-fourth-stage juveniles, and 31% females. All recoveries for the individual incubation intervals were within 7% of these figures. The percent of final recovery density for each stage of *P. brachyurus*, therefore, was approximately the same for all stages after any given incubation interval (Table 2).

At least 88% of the second-stage juveniles of *P. brachyurus* from cotton roots readily passed through a 325-mesh sieve, whereas, 49% of the combined third and fourth-stage juveniles, and 84% of the females were retained on a sieve of this mesh (Table 3). At least 52% of the total population of *P. brachyurus* recovered from cotton roots readily passed through a 325-mesh sieve, whereas, at least 23% passed through a series containing both a 325 and a 400-mesh sieve.

After six months in the greenhouse, *P.*

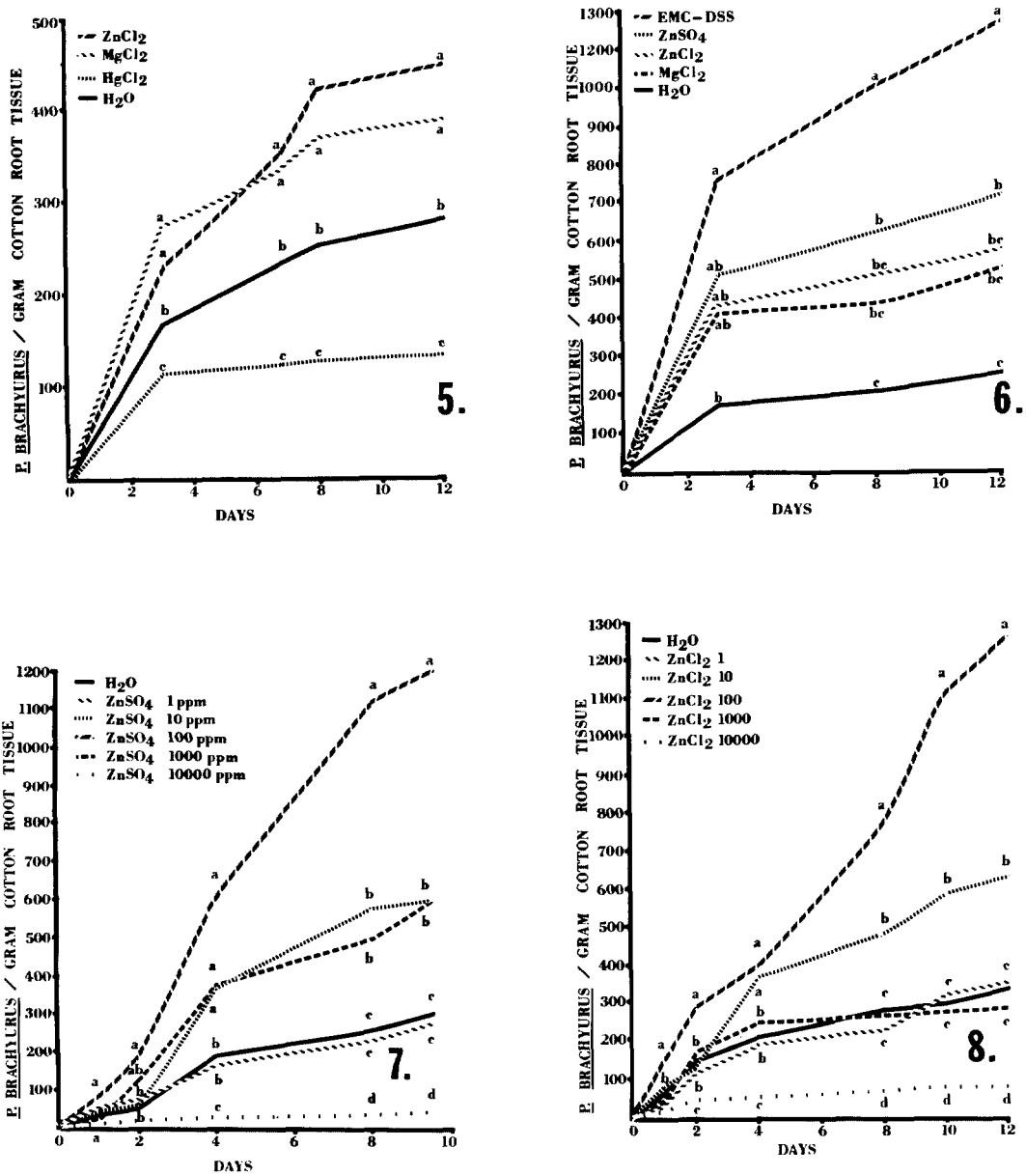
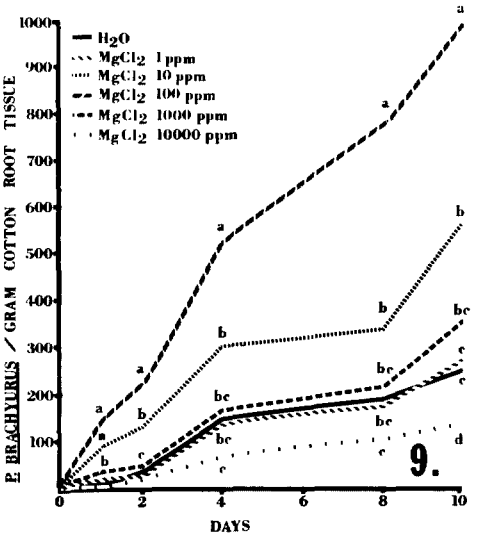


FIG. 5–8. Recovery of *Pratylenchus brachyurus* from cotton roots incubated in various concentrations of incubation solutions. Population densities on a given day marked with same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test. **5.** One hundred ppm zinc chloride, magnesium chloride, or mercuric chloride. **6.** Ten ppm zinc chloride, zinc sulfate, magnesium chloride, or a mixture of 10 ppm ethoxyethyl mercuric chloride (EMC) and 50 ppm dihydrostreptomycin sulfate (DSS). **7.** Five concentrations of zinc sulfate. **8.** Five concentrations of zinc chloride.



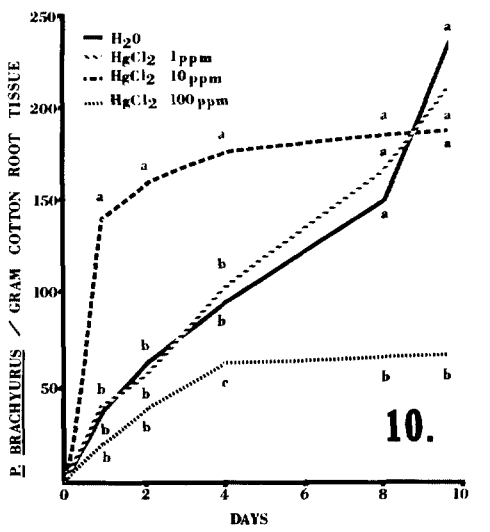


FIG. 9 and 10. Recovery of *Pratylenchus brachyurus* from cotton roots incubated in various concentrations of incubation solutions. Population densities on a given day marked with same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test. **9.** Five concentrations of magnesium chloride. **10.** Three concentrations of mercuric chloride.

brachyurus populations were recovered from both the soil and roots of all plants inoculated with nematodes incubated in 1, 10, 100, and 1,000 ppm DPBA. There was no significant difference between the population densities recovered from the above treatments and those obtained when the roots were incubated in water. *Pratylenchus brachyurus* were not recovered from the soil or roots of plants inoculated with nematodes incubated in 10,000 ppm. Incubation of cotton roots in 10 ppm zinc sulfate, zinc chloride, magnesium chloride, or a 10–50 ppm mixture of EMC-DSS had no ascertain-

TABLE 2. Percent of final recovery density of various life cycle stages of *Pratylenchus brachyurus* extracted from cotton roots after incubation in 100 ppm $ZnSO_4$ for 1, 2, 7, and 14 days.

Stage	Incubation period			
	1 day	2 days	7 days	14 days
Second	5a[1]	16a	90a	98a
Third-fourth	5a	20a	91a	96a
Females	5a	18a	95a	98a
Total	5a	18a	92a	97a

[1] Column means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test.

TABLE 3. Percent of final recovery density of various life cycle stages of *Pratylenchus brachyurus* collected on nested of 325, 400, and 500-mesh sieves, during five cotton root incubation intervals over a period of 21 days.

Screen location and size	Stage			Total population recovered
	Second-stage juveniles	Third-fourth stage juveniles	Females	
Top sieve (325-mesh)	12a[1]	49a	84a	48a
Middle sieve (400-mesh)	30b	36a	15b	29b
Bottom sieve (500-mesh)	58c	15b	1c	23c

[1] Column means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test.

able detrimental effect on the viability of *P. brachyurus.*

Discussion

All of the chemical compounds investigated as components of incubation solutions had an influence on the rate of recovery of *P. brachyurus* from cotton roots, when compared with water. The rate of recovery depended on the concentration of the compound. At optimum concentrations for incubation, the time necessary to recover a certain percent of the population was decreased, whereas, at a higher concentration the time necessary for equivalent recovery was increased. Low concentrations had no influence on the recovery rate.

Dolliver (4) reported that only one third as many *P. penetrans* were recovered from orchard grass roots incubated for three days in 0.1 M potassium nitrate, as were recovered when the roots were incubated in distilled water. These data are in agreement with the present investigation, since 10,000 ppm or 0.06 M zinc sulfate, 0.07 M zinc chloride, and 0.11 M magnesium chloride retarded the rate of recovery, whereas, 100 ppm or 0.6 mM zinc sulfate, 0.7 mM zinc chloride and 1.1 mM magnesium chloride were found to be close to the optimum incubation solution concentrations for these salts. From these data it can be determined that neither the specific cation nor the specific anion of the salts studied was directly responsible for changes in the rate of nematode recovery.

The mechanisms involved in the recovery of migratory endoparasitic nematodes from incubated roots are not well understood. The nematodes could be attracted to the incubation solution, or stimulated to move away from their normal feeding sites, or both. Migratory endoparasites might leave the root at random, or during some specific phase of their life cycle. Increased migration, or stimulation of egg hatch could be possible explanations for the phenomena demonstrated in the present investigation. Various chemicals are known to stimulate egg hatching of *Heterodera* spp., and the emergence of second-stage juveniles from cysts. Clark and Shepard (3) demonstrated that a wide range of inorganic salts stimulated hatching of *H. schachtii* eggs. Concentrations of zinc chloride and zinc sulfate optimum for hatching of *H. schachtii* eggs were three to four times greater than the optimum concentrations for the recovery of *P. brachyurus.* Whitney and Doney (10) reported that EMC and DSS stimulated hatching of *H. schachtii* eggs. It is possible that the more rapid rate of recovery of *P. brachyurus* from cotton roots incubated in various solutions, resulted from a stimulation of egg hatching.

At all incubation intervals during the first three weeks after roots were removed from soil, the population density consisted of approximately 25% second-stage juveniles, 44% third-fourth-stage juveniles, and 31% females. The percent recovery of the various stages depended on the screening technique employed. Use of a single 325-mesh sieve was not adequate for the recovery of a majority of the second and third-fourth-stage juveniles, although 84% of the females collected were retained on a sieve of this mesh.

Incubation solutions containing 10 ppm EMC and 50 ppm DSS, 10, 100, or 1,000 ppm DPBA, or 10 ppm various salts had no apparent influence on the viability of *P. brachyurus.* Whitney and Doney (10) found that *H. schachtii* viability was not retarded by similar incubation solutions used to stimulate egg hatch.

Diagnosis of nematode diseases of higher plants is only as accurate as the techniques employed in the qualitative and quantitative analysis of nematode populations. For example, approximately 90% of the recover-

able *P. brachyurus* can be obtained within 96 hrs when cotton roots are incubated in a mixture of 10 ppm EMC and 50 ppm DSS, whereas using water as the incubation solution, more than two weeks are necessary for recovery of an equivalent percentage of the population. In the development of more efficient and accurate techniques for the diagnosis of nematode diseases of plants, it will be essential to have additional information on all factors affecting the recovery of nematodes from soil and host tissue.

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