Temperature Effects on Reproduction and Pathogenicity of *Pratylenchus coffeae* and *P. brachyurus* and Survival of *P. coffeae* in Roots of *Citrus jambhiri*

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Abstract: Optimum temperature for reproduction of *Pratylenchus coffeae* on Rough lemon, *Citrus jambhiri* Lush. was 29.5 C. *Pratylenchus coffeae* populations reached 7653 per g of root 2 months after initial inoculation with 140 nematodes. Maximum plant weight reduction was 38%; root weights were reduced by as much as 47%. *Pratylenchus brachyurus* significantly reduced plant and root weights but population sizes never approached those of *P. coffeae*. *Pratylenchus coffeae* survived in stored excised roots and were still infective after 4 months at temperatures ranging from 4.5 to 32 C. *Key Words:* Constant temperatures, Storage survival, Recovery.

In 1963 Gotoh and Ohshima (4) found *Pratylenchus coffeae* (Zimmerman) Filipjev and Schuurmans Stekhoven associated with citrus (*Poncirus trifoliata* [L.] Raf.) in Japan. In 1966 Yokoo and Ikegemi (7) reported that *P. coffeae* severely damaged citrus and other crops in the warmer regions of Japan. Yokoo and Kukoda (8) also reported temperature control tests and field observations which indicated 25–28 C optimum for invasion and reproduction of *P. coffeae* on potato. Their observations were mostly speculative, since the host plants in the controlled temperature studies died except at 8 and 20 C. O'Bannon and Tomerlin (5) inoculated 6-month-old greenhouse-grown Rough lemon (*Citrus jambhiri* Lush.) seedlings with *P. coffeae* and *P. brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven and assayed the populations at 6-week intervals for 16 months. *Pratylenchus coffeae* populations peaked at 10,000 nematodes per g of root after 9 months at ambient greenhouse temperatures; populations of *P. brachyurus* reached 100 nematodes per g of root after 13 months and caused no measurable growth reduction. Shoot growth of plants infected with *P. coffeae* had decreased 22% 16 months after soil infestation. In the same studies, they found that soil infestation initiated in summer favored maximum population development of *P. coffeae*.

In India, Siddiqi (6) found *P. coffeae* on *C. limon*, *C. reticulata* Blanco, *C. sinensis* (L.) Osbeck, and *Musa paradisiaca* L. His studies showed that young Rough lemon seedlings inoculated with 600 *P. coffeae* were stunted after 4 months. No temperature data were reported.

Several reports have indicated *Pratylenchus* spp. parasitic upon, or associated with citrus, but only *P. brachyurus* (1) and *P. coffeae* (5) have been proven pathogenic. Feldmesser and Hannon (3) inoculated *C. limon* with an undetermined number of *P. coffeae* but reported an increase in numbers of nematodes associated with stunted and deteriorating root systems. No quantitative measurements of the virulence or extent of *P. coffeae* population increase were given, however. They also reported that a mixture of *P. zeae* Graham, *P. coffeae*, *P. brachyurus* and *P. penetrans* (Cobb) Filipjev and Schuurmans Stekhoven significantly reduced root and top weights of citrus seedlings and

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concluded that members of this genus were a hazard to citrus in Florida.

The purposes of this paper were to define temperature effects on reproduction and pathogenicity of *P. coffeae* and *P. brachyurus* on Rough lemon (*Citrus jambhiri* Lush.) seedlings and to determine the survival potential of *P. coffeae* in desiccated and moist fallow soil.

Materials and Methods

Temperature: Three experiments on the effects of temperature on infection and reproduction of *P. coffeae* and *P. brachyurus* on Rough lemon seedlings were conducted in temperature tanks maintained at 24, 26, 29, 32 and 35 C ± 1 C. Seedlings grown in steam-pasteurized sand to the second and third true leaf stage were individually transplanted into plastic containers containing a Lakeland fine sand (sand, silt and clay content, 97, 0 and 3%, respectively) with an organic content of 0.13% and a moisture equivalent of 1.2%. The volume of the plastic containers used in Tests 1 and 2 was 946 ml; those in Test 3 were 1900 ml. Plants were maintained on a greenhouse bench 10–14 days before inoculation and transferred to controlled temperature tanks. The nematode inoculum used in these studies was obtained from infected citrus roots. *P. coffeae* inoculum came from citrus seedlings maintained in a greenhouse, and *P. brachyurus* from roots of an infected tree in a citrus grove containing a pure culture of *P. brachyurus*. Inoculum was introduced either in water suspension (containing 4 ppm of ethoxyethyl-mercury chloride) in 3 holes in the soil around the base of the plant or in finely chopped infected roots (3 g/20 cm pot). One gm of roots was added to each of 3 holes in the soil around the base of each plant. Twenty-four hours after infestation, containers with the two types of inoculum (Test 1) or nematode species (Tests 2 and 3) were randomized in their respective temperature tanks. Treatments were replicated 5–15 times. Durations of the three tests were 7, 8, and 12 weeks, respectively.

At harvest, seedlings were gently removed from the containers, the roots washed, and plants weighed. Roots were excised from the plants, weighed, cut into 5 mm lengths and incubated in pint jars at 21 C (9). Nematodes which emerged from the roots were counted on the 3rd, 5th, and 7th day after being placed in the jars. The total counts of the readings are presented in Table 1. One hundred *P. coffeae* collected from seedlings at each temperature were selected at random and the proportions of females, males, and larval (L_2, L_3, and L_4) stages determined.

Moisture: *Pratylenchus coffeae* inoculum used to study survival in dry and moist fallow soil was obtained from naturally-infested Rough lemon roots. Feeder roots were cut from main roots, thoroughly mixed, cut into lengths < 1 cm and remixed. Then 0.75 g of the chopped roots were placed in each of 360 7-cm plastic petri dishes containing 20 g of oven dried Lakeland fine sand. Dishes were randomly distributed into lots of five, placed in plastic bags and sealed. A comparable 360-dish group was set up containing soil with moisture content approximately at field capacity. All dishes were individually shaken to distribute the roots in the soil. Forty dishes (eight bags) of each soil (dry or moist) were incubated at 5, 10, 16, 21, 26, 32, 43, and 49 C. Five dishes each of dry and moist soil were removed from each temperature chamber biweekly for 16 weeks. Soil was washed from the roots and the roots incubated in the dishes and the nematodes were extracted and counted. Infectivity of nematodes recovered from each temperature at the final sampling were checked by inoculating roots of Rough lemon seedlings.

TABLE 1. Numbers of *P. coffeae* or *P. brachyurus* extracted from roots of Rough lemon grown at five temperatures.

Test No.	Test duration	Nematode species	Inoculation method	Inoculation level	Pratylenchus/g of root 24 C	26 C	29.5 C	32 C	35 C
1	7 wks	*P. coffeae*[a]	chopped roots	740	150	—	1640	—	20
		P. coffeae[a]	water suspension	496	248	—	2090	—	8
2	8 wks	*P. coffeae*[b]	water suspension	140	456	4160	7653	5020	—
		P. brachyurus[c]	water suspension	140	3	160	240	740	—
3	12 wks	*P. coffeae*[c]	water suspension	200	—	466	1479	1100	0
		P. brachyurus[c]	water suspension	200	—	48	104	58	4

[a] Means represent 15 replications.
[b] Means represent 5 replications.
[c] Means represent 10 replications.

RESULTS AND DISCUSSION

TEMPERATURE: *Pratylenchus coffeae* increased under constant temperatures from 26–32 C (Table 1). The maximum population increase occurred at 29.5 C, which was slightly higher than what Yokoo and Kukoda had speculated (8). O'Bannon and Tomerlin (5) obtained 10,000 *P. coffeae* per g of root after 9 months at ambient greenhouse temperatures. Under controlled conditions we obtained 7653 per g of root in 2 months (Table 1). It is doubtful that reproduction occurred at 35 C, nematodes recovered in Tests 1 and 3 probably survived from the original inoculum. Some reproduction occurred at 24 C. Field samplings from infected trees in Florida indicate that reproduction continues at temperatures as low as 18 C.

TABLE 2. Effects of *Pratylenchus coffeae* and *P. brachyurus* on growth of Rough lemon seedlings at different soil temperatures, Test 2.

Nematode		Soil temp. (C)	Mean plant wt. at end of test (g)	Plant wt. reduction (%)	Mean root wt./plant (g)	Root wt. reduction (%)
P. coffeae	Treatment	24	4.7	22	2.5	29
	Check		6.0		3.5	
	Treatment	26	3.8	38	1.7	47
	Check		6.1		3.2	
	Treatment	29.5	4.7	30	1.8	47
	Check		6.7		3.4	
	Treatment	32	4.9	30	2.5	34
	Check		6.9		3.8	
P. brachyurus	Treatment	24	4.6	23	2.4	27
	Check		6.0		3.3	
	Treatment	26	5.1	16	2.1	32
	Check		6.1		3.1	
	Treatment	29.5	5.9	6	2.8	15
	Check		6.3		3.3	
	Treatment	32	4.5	35	2.2	44
	Check		6.9		3.9	

TABLE 3. Temperature effects on per cent distribution of life cycle stages of *Pratylenchus coffeae* recovered from *Citrus jambhiri* roots after 8 weeks.

Temp. (C)	Life stages				
	L_2	L_3	L_4	♀♀	♂♂
24	3	48	15	21	13
26	3	33	16	34	14
29.5	3	35	24	24	14
32	5	30	24	20	21

In Test 1, results in population build-up was similar where the two different inoculation techniques were used (Table 1). At the termination of the second and third tests, *P. coffeae* increased to very high numbers in a relatively short time, but populations of *P. brachyurus* reached only 14% and 7% that of *P. coffeae*, respectively.

Plant response to nematode infection was similar in all three experiments. Therefore, data on the second experiment only are presented (Table 2). *P. coffeae* significantly reduced total plant weight as much as 38% over the uninoculated checks. Total plant weight correlated inversely with numbers of *P. coffeae* recovered per gram of root. *Pratylenchus brachyurus* reduced plant weights even though populations did not increase as much as *P. coffeae*. These findings agreed with those of Brooks and Perry (1), who found that *P. brachyurus* populations only doubled in a 6-month period, but that even relatively low populations of *P. brachyurus* were definitely pathogenic to small sour orange seedlings (*Citrus aurantium* L.). Under optimum field conditions, *P. coffeae* probably would increase faster and be detrimental to Rough lemon rootstocks sooner than *P. brachyurus*.

The proportion of the various *P. coffeae* life stages appears to be constant regardless of the temperature (Table 3). A greater number were in the third larval stage (L_3) than any other.

TABLE 4. Numbers of *P. coffeae* recovered from Rough lemon roots after 8 storage periods at 9 temperatures in moist and dry soils.

Temp. (C)		Storage period in weeks							
		2	4	6	8	10	12	14	16
49	Dry	0	0	0	0	0	0	0	0
	Wet	0	0	0	0	0	0	0	0
43	Dry	1	2	0	1	0	0	0	0
	Wet	8	0	0	0	0	0	0	0
38	Dry	1	0	0	0	0	0	0	0
	Wet	8	0	0	0	0	0	0	0
32	Dry	35	9	6	3	2	1	2	0
	Wet	324	149	126	128	101	7	12	120
27	Dry	14	59	31	48	16	6	6	17
	Wet	121	335	143	66	66	66	35	260
21	Dry	41	141	32	71	8	19	21	260
	Wet	61	20	16	5	86	4	1	12
16	Dry	36	51	44	46	18	18	5	20
	Wet	32	234	90	48	5	22	10	150
10	Dry	9	40	80	28	5	8	66	320
	Wet	16	122	64	61	1	147	110	960
4.5	Dry	14	6	32	3	1	1	2	15
	Wet	34	62	49	25	2	1	1	1

MOISTURE: *P. coffeae* did not survive extended storage in either moist or dry soils at 38 C (Table 4). With minor exceptions, nematodes survived best in infected roots stored in soils with initial moisture content near field capacity. At the last sampling, nematodes extracted from roots stored at all temperatures, from either wet or dry soils, infected Rough lemon seedlings. All stages of *P. coffeae* survived equally well in storage. This was demonstrated by dissecting eggs, all larval stages, males and females from the roots at each temperature. *P. coffeae* can survive in small feeder roots for extended periods of time under the most adverse Florida conditions. Studies reported by Feldmesser and Rebois (2) showed that *P. brachyurus* also can survive adverse conditions.

Although *P. coffeae* is not known to be widespread in Florida citrus, the results

reported here show that it is a potentially serious threat to this crop.

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