

sured from photographs of 155 selected nematodes and nematode weights were calculated according to Andrásy's (1956) formula :

$$Wt = (W^2 \times L)/(16 \times 10^5)$$

where Wt = fresh weight in μg , W = greatest width in μm , and L = length (μm) excluding the tail. Nematode developmental stages were separated by size. In order to calculate the amount of energy consumed by nematodes, fresh weights were converted into calories on the basis of 2.152 calories per mg fresh weight and dry weights as 25 % of fresh weights (Yeates, 1979). Energy consumed by nematodes is defined as the amount of energy equivalent to a nematode size.

Each of 24, 8-month-old potted *Pinus sylvestris* L. seedlings were treated with either wound, wound and water, wound and *B. xylophilus* culture filtrate, and wound and 400, 4000 or 40 000 *B. xylophilus*, or unwounded checks as described by Melakeberhan and Webster (1990b). With the exception of the wound and check, all treatments received a 75 μl aliquot with or without nematodes, and all pines were arranged randomly in a growth chamber at 25 °C (Melakeberhan & Webster, 1990b).

The pine seedlings were examined daily and disease symptoms recorded. When pines died (Melakeberhan & Webster, 1990b), they, together with three or four symptomless (healthy) pines from all treatments, were harvested. This was done so as to allow comparison of dry matter accumulation, the parameter selected to test the hypothesis, between and within treatments. Shoots were dissected longitudinally, vascular necrosis noted, fresh weights recorded and cut into lengths of approximately 1 cm. All of the plant tissue except for 0.2 g of the shoot fresh weight was oven-dried for 24 h at 75-80 °C and dry weights recorded.

Nematode populations in pine seedlings were estimated after 24 h of extraction on a modified Baermann funnel of 0.2 g shoot fresh weight sub-samples (Melakeberhan & Webster, 1990b). Nematode developmental stages were not separately counted. Hence, the mean weights and calorific equivalents of all developmental stages and both sexes were used for calculations of energy consumed by nematodes.

Changes in dry matter accumulation up to 37 days after inoculation, or up to the time of recorded plant death for each treatment, were recorded. Nematode dry weights were subtracted from that of the host (Melakeberhan & Ferris, 1989). Host dry matter was converted into calories based on 4.7 kcal per g dry weight (Leith, 1968). Calculations of differences in dry matter accumulation between nematode-infected dead and symptomless pines and control treatments were similar to those described by Melakeberhan and Ferris (1989). Total dry matter accumulation of dead and symptomless nematode-infected plants, with or without nematodes, were compared with all the controls.

Regression and ANOVA methods were used to analyze the data (Anon., 1985).

Results

The range of nematode width and length from the second-stage juvenile to the adult was 5-28 μm and ca 200-1000 μm (Fig. 1) with fresh weight values of 0.02-0.50 μg (Fig. 2), respectively. Because of the degree of overlapping between the sexes, a mean of 0.1499 μg fresh weight per nematode was used for energy equivalent calculations. The corresponding values for dry weights and calorific equivalents from the second-stage juvenile to the adult (which are direct conversions of fresh weight) were 0.005-0.13 μg and 0.000043-0.0011 calories, with means of 0.03778 μg and 0.000305 calories per nematode, respectively.

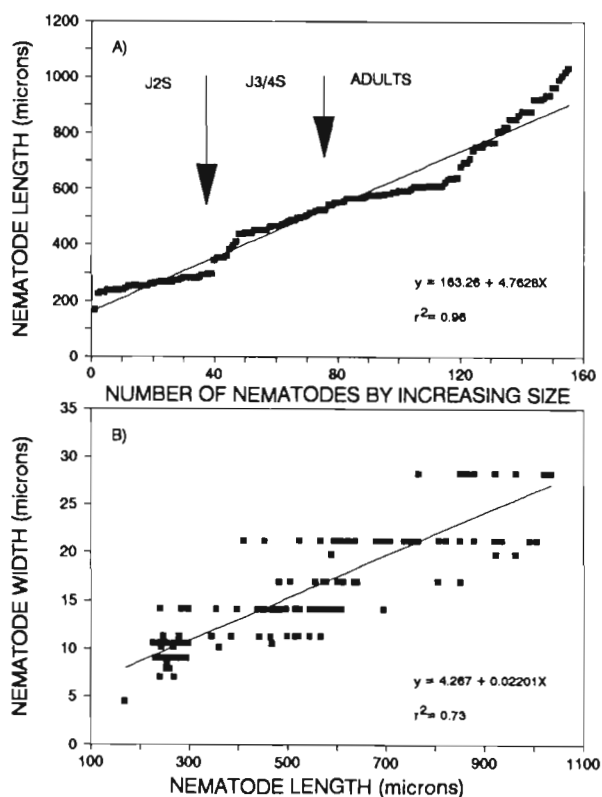


Fig. 1. The relationship between (A) developmental stage and body length and (B) body length and width of 155 nematodes *Bursaphelenchus xylophilus*. Changes in nematode developmental stage, as it relates to length, is shown by arrows.

Pine death was preceded by chlorosis and wilting, and dead pines almost invariably had ca a 2-4 cm long necrotic lesion in the vascular tissue around the inoculation sites. Symptomless pines from the medium and high nematode inoculum treatments had necrotic vascular lesions but not as severe as in the dead pines. At 7, 10 and 17 days after inoculation, three, three, and one

Table 1. Comparison of total dry matter accumulated (kcal) by the check (C), wound (WD), wound and water (WDW) and wound and *Bursaphelenchus xylophilus* culture filtrate (WDCF) with increasing number of nematodes extracted per pine, and with (+) or without (-) nematodes on dead pines at 17 days and on symptomless pines at 17, 30 and 37 days after inoculation. x = comparison does not include the highest nematode treatment (see Methods). There was no significant difference between any of the treatments.

Days after inoculation	C		WD		WDW		WDCF	
	+	-	+	-	+	-	+	-
DEAD								
17	9.44	9.48	9.31	9.97	9.46	9.50	9.81	9.85
SYMPTOMLESS								
	+	-	+	-	+	-	+	-
17	9.00	9.00	9.32	9.32	9.01	9.01	9.24	9.24
30	9.79	9.79	9.48	9.48	9.25	9.25	9.26	9.26
37	10.08	9.74	9.58	9.23	9.26	8.91	9.04	8.69

There was no significant difference in dry matter accumulation between *B. xylophilus*-infected (dead or symptomless) and non-infected pines. If the working hypothesis is true, *i*) *B. xylophilus*-infected pines should have had smaller biomass than uninfected controls of equivalent age and development, *ii*) nematode food consumption should have accounted for the difference in the amount of dry matter accumulation between infected and uninfected pines, and *iii*) pines may or may not have died. However, the amount of energy consumed by *B. xylophilus* accounted for less than 4 calories (Fig. 4) or ca 0.0001 % of the total dry matter accumulated by the pines (Table 1) despite pine seedling mortality of 30-50 % (Fig. 3). Since there was no difference in biomass accumulation between nematode-infected and uninfected pines but highly infected pines died, the data suggest that the hypothesis is not true and show that *B. xylophilus*-infected pine seedlings die due to causes other than energy consumed by the nematodes. This pattern contrasts with the *M. incognita* infection of grape where both the nematode energy demand and the effect on host physiology are important in host response (Melakeberhan & Ferris, 1989) and in the *M. incognita*-grape situation the host plant does not die.

In conclusion, measuring the increase in nematode biomass allows for the calculation of *B. xylophilus* weights and energy equivalence and for the separation of the nematode food requirements from the effect on host physiology. This helps our understanding of how *B. xylophilus* kills its host. Food demand of the *B. xylophilus* population does not seem to be a significant factor in causing the death of young pine seedlings. Rather, the *B. xylophilus* population appears to influence P

induced host metabolic modifications that result in altering water relations (Tamura *et al.*, 1987; Kuroda, 1989; Ikeda *et al.*, 1990; Melakeberhan *et al.*, 1991) which, in turn, affect other related physiological processes (Melakeberhan & Webster, 1990a).

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