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Temperature-mediated Behavioral Relationships in *Bursaphelenchus xylophilus, B. mucronatus,* and Their Hybrids[1]

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Abstract: The influence of temperature on reproduction and movement was examined for seven geographic isolates of *Bursaphelenchus xylophilus,* three of *B. mucronatus,* and two of their interspecific hybrids. All nematode isolates tended to be more active and fecund the higher the temperature, with the isolates of *B. xylophilus* reaching a reproductive peak at higher temperatures than isolates of *B. mucronatus.* Most isolates of *B. xylophilus* and *B. mucronatus* did not produce significantly more progeny at higher male-to-female ratios. The interspecific hybrids appear to possess temperature-related characteristics of either *B. xylophilus* or both of the parents.

Key words: behavior, *Bursaphelenchus mucronatus, B. xylophilus,* fecundity, interspecific hybrids, nematode, sex ratio.

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934), Nickle 1970, and *B. mucronatus* Mamiya & Enda 1979 are members of the pinewood nematode species complex (PWNSC) (20). The wilting caused by *B. xylophilus* in susceptible pines (12) results in serious economic damage to Japanese pine forests (10). Since its probable introduction from North America around the turn of the century, *B. xylophilus* has spread throughout most Japanese forests, but wilting is not induced in Japan in cool areas or locations above 700 m elevation (10). *Bursaphelenchus mucronatus* is believed to be native to Japan and is widespread, but it is not associated with epidemic pine wilt disease (12). Isolates of *B. mucronatus* from France (4,18) and Norway (18) kill pine seedlings under experimental conditions at temperatures above 23 C. Despite the widespread distribution of *B. xylophilus* in North America (16) and *B. mucronatus* in Europe (5,8), nematode-induced wilting is rare outside of Japan; in North America it is confined to nonindigenous trees in regions with a July mean daily temperature above 20 C (17).

The severity of disease symptoms and tree mortality can be correlated with the number of nematodes inoculated (11,14). The development of wilt symptoms at high temperatures depends on the degree of nonreversible tracheid cavitation caused by nematode damage (6), and the number of cells disrupted correlates well with the number of nematodes present in a tree (7). Futai (3) reported that Japanese isolates of *B. xylophilus* developed more rapidly and produced more offspring at all temperatures than did Japanese isolates of the less pathogenic *B. mucronatus.*

Bursaphelenchus xylophilus is amphimictic, and continual copulation is necessary for maximum offspring production (9). The purpose of our research was to investigate the effect of temperature on movement and reproductive rate of various isolates of *B. xylophilus* and *B. mucronatus* and two of their hybrids. Also, by varying the sex ratios under specified experimental conditions, we examined the effect of increased mating probability on fecundity.

MATERIALS AND METHODS

Three isolates of *B. mucronatus,* seven isolates of *B. xylophilus,* and two of their interspecific hybrids (Table 1) were maintained monoxenically on *Botrytis cinerea*

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TABLE 1. The number† of complete sine waves/min for adult male *Bursaphelenchus xylophilus*, *B. Mucronatus*, or their interspecific hybrids at 10, 20, and 30 C.

	Temperature		
Nematode isolates‡	10 C	20 C	30 C
	B. xylophilus		
MsP4 (R. I. Bolla)	12.7 ± 1.4	36.1 ± 4.2*	83.5 ± 6.1*
Ibaraki (Y. Mamiya)	12.9 ± 1.1	30.0 ± 2.3*	60.8 ± 4.5*
B.C. (R. V. Anderson)	17.4 ± 1.4	31.2 ± 2.7*	64.9 ± 5.1*
Q52A (R. V. Anderson)	19.1 ± 1.1	33.8 ± 3.1*	64.7 ± 5.0*
St. John (R. V. Anderson)	12.2 ± 0.7	38.7 ± 2.8*	58.3 ± 3.4*
St. William (R. V. Anderson)	13.1 ± 0.7	32.6 ± 1.4*	50.0 ± 6.3*
BXUJA (R. I. Bolla)	6.3 ± 0.2	27.4 ± 1.4*	60.5 ± 5.5*
	B. mucronatus		
Chiba (Y. Mamiya)	7.8 ± 0.6	38.5 ± 1.6*	73.5 ± 6.2*
French (G. de Guiran)	9.8 ± 0.7	30.1 ± 1.5*	71.8 ± 6.8*
Norway (D. G. McNamara)	15.5 ± 0.8	34.0 ± 3.1*	48.8 ± 4.4*
	Interspecific hybrids		
Chiba ♂ × St. William ♀	17.7 ± 1.9	35.5 ± 2.9*	48.8 ± 2.8*
Chiba ♂ × St. John ♀	14.4 ± 1.5	24.7 ± 1.0*	35.3 ± 2.7*

† Each value is the mean of eight replicates ± SE.
‡ Source in parenthesis; see also (19) for details.
* These values are significantly different ($P < 0.05$) from each other and from the 10 C values.

cultured on 1% potato-dextrose agar (PDA) (3). Nematodes for experimental purposes were rinsed from either the fungal mats or the culture dish lids with distilled water. Hybrids between isolates were produced by placing a virgin, fourth-stage female juvenile of one isolate together with a mature male of another on a 3-cm-d *B. cinerea* plate at 27 C. Both parents were removed from the plate as soon as the first eggs were seen, and the resulting inbred culture of the hybrid cross was maintained on PDA.

Movement rates were determined for eight adult males of each isolate and hybrid at 10, 20, or 30 C, on thin-film PDA plates obtained by rinsing fresh, 9-cm-d plastic petri dishes with warm (45 C), sterile PDA and immediately pouring out the excess agar. The complete sine waves (forward or reverse) made by the nematodes on the agar over a 1-minute period were counted by direct observation through a dissecting microscope. Nematodes were allowed to equilibrate on the plates at the desired temperature for 1 hour before observation.

Reproductive rates of the isolates and hybrids were determined as follows. Two pairs of adult male and female nematodes were placed on each of five 6-cm-d PDA plates inoculated 7 days previously with *B. cinerea*. Offspring production per day was determined by extracting nematodes with a Baermann funnel at room temperature from cultures that had been maintained at temperatures of 21.5, 24.5, 27.5, and 30.5 C over periods of 7, 4, 3, and 2 days, respectively. This procedure ensured that the offspring counted were only the F_1 generation.

Sex ratio effects on the reproductive rates of the isolates and their hybrids were determined by varying the number of males available to each female from 0.5 to 4 males/female. Five 6-cm-d petri plates of *B. cinerea* (prepared as before) were used for each isolate or hybrid; each plate contained two adult females and from one to eight adult males. Offspring were counted after incubation for 3 days at 27.5 C.

The data were analyzed with a single-factor analysis of variance and Scheffé's multiple comparison test (2).

RESULTS

All isolates moved faster the higher the temperature, regardless of their geographic origin. Like their *B. xylophilus* parents, the interspecific hybrids showed a tendency to move faster at 10 C than did their *B. mucronatus* Chiba parent (Table 1).

All *B. xylophilus* isolates, with the exception of Q52A, produced more ($P < 0.05$) offspring at 27.5 C or greater. The *B. mucronatus* isolates produced most offspring at 27.5 C or lower temperatures. Both interspecific hybrids produced most progeny at 27.5 C (Table 2).

There was no significant difference in the number of offspring produced at different sex ratios for most isolates of *B. xylophilus*, with the exception of Q52A and BXUJA, which produced more ($P < 0.05$) offspring at higher sex ratios (Table 3). The *B. mucronatus* French isolate produced significantly more offspring at a 1.0 male:female ratio ($P < 0.05$). Only one of the interspecific hybrids, the Chiba male × St. John female, produced significantly more offspring at 2.0 and 4.0 male:female ratios ($P < 0.05$); this hybrid appears to possess reproductive characteristics of the *B. xylophilus* St. John parent (Table 3).

DISCUSSION

The activity and fecundity of isolates of the two *Bursaphelenchus* species and of two of their interspecific hybrids is greater the higher the temperature. A reason for the greater pathogenicity of *B. xylophilus* than *B. mucronatus* in the field may be the tendency for this species to move more rapidly than *B. mucronatus* at lower temperatures and thereby cause cellular damage, which under subsequent high temperatures makes the tree more susceptible to wilting and water stress. Pine seedling pathogenicity caused by the Norwegian and French *B. mucronatus* isolates (18) may well be attributable to the large inoculum levels or substantial nematode reproduction with subsequent conductive tissue damage.

Differences in pathogenicity of several *B. xylophilus* isolates to pine trees have been reported (1), but comparisons among isolates and their hybrids are difficult because of the variety of inoculation techniques and experimental conditions. It has been reported that *B. xylophilus* isolates are more pathogenic to pine trees and seedlings than are *B. mucronatus* isolates (5,12,18) and that *B. xylophilus* intraspecific hybrids are more pathogenic than *B. mucronatus* isolates (15). In addition, *B. xylophilus* and *B. mucronatus* interspecific hybrids were reported to be more pathogenic than the *B. mucronatus* parental isolates and as pathogenic as *B. xylophilus* parental isolates, having inherited their virulence from the *B.*

TABLE 2. Mean number† of offspring produced per day by two pairs of adult *Bursaphelenchus xylophilus*, *B. mucronatus*, or their interspecific hybrids reared on *Botrytis cinerea* at different temperatures.

	Temperature			
Nematode isolate	21.5 C	24.5 C	27.5 C	30.5 C
		B. xylophilus		
MSP4	7.5 ± 1.8	5.4 ± 0.6	13.4 ± 4.2	20.6 ± 3.8*
Ibaraki	5.2 ± 0.7	6.1 ± 2.8	12.5 ± 3.5	15.8 ± 3.9*
B.C.	4.6 ± 1.0	3.6 ± 0.6	6.3 ± 0.6	30.7 ± 7.9*
Q52A	5.4 ± 1.4	7.8 ± 1.9	11.6 ± 4.0	7.4 ± 2.6
St. John	7.9 ± 2.3	12.0 ± 3.7	10.6 ± 2.0	17.7 ± 5.3
St. William	6.4 ± 1.7	4.9 ± 1.3	10.2 ± 2.6	27.8 ± 8.5*
BXUJA	2.7 ± 0.3	2.7 ± 0.6	17.8 ± 3.6*	21.5 ± 6.0*
		B. mucronatus		
Chiba	0.8 ± 0.3	5.2 ± 0.9*	5.6 ± 0.7*	4.8 ± 1.2*
French	8.5 ± 1.7	19.0 ± 4.4*	19.1 ± 3.9*	6.0 ± 0.7
Norway	2.5 ± 1.0	5.1 ± 0.7	10.4 ± 1.9*	2.5 ± 0.5
		Interspecific hybrids		
Chiba ♂ × St. William ♀	3.2 ± 1.9	7.3 ± 1.1	11.2 ± 3.4*	6.0 ± 2.0
Chiba ♂ × St. John ♀	5.8 ± 1.6	10.1 ± 3.1	23.3 ± 3.3*	18.5 ± 1.6*

† Each value is the mean of eight replicates ± SE.

* These values are significantly different ($P < 0.05$) from each other and from the rest of the values.

TABLE 3. Mean number† of offspring produced per day by two females of isolates of *Bursaphelenchus xylophilus*, *B. mucronatus* or their interspecific hybrids after exposure to males at four sex ratios on *Botrytis cinerea* at 27.5 C.

Nematode isolate	Sex ratio‡ 0.5	 1.0	 2.0	 4.0
		B. xylophilus		
MSP4	10.8 ± 1.4	13.5 ± 4.2	16.3 ± 6.1	22.7 ± 3.5
Ibaraki	5.7 ± 1.1	12.5 ± 2.3	13.5 ± 4.5	14.6 ± 2.9
B.C.	4.8 ± 1.4	6.3 ± 2.7	7.9 ± 5.1	10.3 ± 2.0
Q52A	6.2 ± 1.1	11.6 ± 3.1	18.2 ± 5.0	28.4 ± 9.8*
St. John	16.0 ± 0.7	21.4 ± 2.8	18.6 ± 3.4	23.0 ± 3.6
St. William	18.0 ± 0.7	16.3 ± 1.4	10.2 ± 6.3	18.0 ± 2.3
BXUJA	11.1 ± 0.2	17.8 ± 1.4	22.0 ± 5.5*	25.3 ± 4.3*
		B. mucronatus		
Chiba	5.1 ± 0.6	3.9 ± 1.6	7.9 ± 6.2	5.4 ± 1.0
French	15.2 ± 0.7	27.8 ± 1.5**	13.7 ± 6.8	19.7 ± 5.1
Norway	16.1 ± 0.8	13.1 ± 3.1	12.0 ± 4.4	10.5 ± 4.2
		Interspecific hybrids		
Chiba ♂ × St. William ♀	8.3 ± 1.9	11.2 ± 2.9	10.5 ± 1.8	8.1 ± 2.2
Chiba ♂ × St. John ♀	9.4 ± 1.5	23.3 ± 1.0	27.9 ± 2.7*	37.7 ± 9.9*

† Each value is the mean of five replicates ± SE.

‡ Male to female.

* These values are significantly different ($P < 0.05$) from the 0.5 male/female ratio values.

** This value is significantly different ($P < 0.05$) from the 2.0 male/female ratio value.

xylophilus parent (15). The present study suggests that interspecific hybrids possess temperature-related characteristics of either *B. xylophilus* or both *B. xylophilus* and *B. mucronatus*. However, Schauer-Blume (18) showed that a Norwegian isolate of *B. mucronatus* was more pathogenic to seedlings of *Pinus sylvestris*, at continuous temperatures of 23 ± 2 C, than either a French or Japanese isolate of *B. mucronatus*, and that disease development and seedling damage caused by the Norwegian isolate was similar to that caused by *B. xylophilus*.

Despite interisolate variability, it would be reasonable to expect that, in general, higher temperatures would cause greater nematode activity and, at satisfactory population densities and sex ratios, would result in greater fecundity and population increases. Larger numbers of nematodes are likely to cause more tissue and physiological (13) damage and result in more rapid and severe wilt in susceptible trees than few nematodes, especially when the trees are stressed.

Caution should be used in extrapolating data obtained with nematodes from *B. cinerea* cultures to the situation occurring in pine trees. It is known, for instance, that *B. xylophilus* reproduces more rapidly on fungus than on pine callus (19). Nevertheless, because of the difficulty of carrying out experiments with mature pines in the field, working hypotheses must be developed in the laboratory and used to further understanding of the biology of these remarkable pathogens. The results presented here are not believed to be diet specific. They show some behavioral differences among *B. xylophilus*, *B. mucronatus*, and hybrids, and they provide an additional perspective on the possible role of temperature-mediated behavior in pathogenesis.

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