Some Effects of Temperature on Xiphinema americanum and Infection of Cucumber by Tobacco Ringspot Virus

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ABSTRACT

Xiphinema americanum acquired tobacco ringspot virus (TRSV) at 16, 22, 28, and 34 C, and transmitted at all temperatures except 16 C. The optimum temperature for acquisition and transmission was 28 C. After mechanical inoculation of cotyledons and symptom development, the titer of TRSV in the roots of cucumber did not differ greatly during a subsequent 10-day period at 16, 22, or 28 C, but it did decrease steadily at 34 C. Some cucumber plants grown at 16, 22, 28, and 34 C became infected after mechanical inoculation of roots with TRSV. Surviving nematodes recovered after 10-days of feeding access to cucumber ranged from 39-52% at 16, 22, and 28 C, but only 5% at 34 C. Poor survival of nematodes seems to be the sole reason for low levels of virus transmission at 34 C. Nematodes moved greater distances in sand as the temperature increased from 16 to 28 C. Some nematodes had moved at least 15 cm after 10 days at 16 and 22 C, and 17.5 cm at 28 C. Nematodes were attracted toward cucumber plants at 28 C, and to a lesser extent at 22 C. There was no indication of attraction toward plants at 16 C. It is postulated that differences in attractiveness of cucumber as a feeding host of *X. americanum* account for lower rates of acquisition and transmission of TRSV at temperatures below 28 C.
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Previous experiments in this laboratory (3, 4, 11, 12, 14) on transmission of tobacco ringspot virus (TRSV) by *Xiphinema americanum* Cobb were conducted at 28-30 C soil temperature because results of earlier tests suggested that this was the optimum temperature. McGuire (11) has shown that *X. americanum* is an effective vector of TRSV under these conditions, particularly in transmission from cucumber to cucumber. The effect of temperature could be on the virus-vector-host interaction or on the individual organisms involved.

Activity of *X. americanum* has been shown to differ at different temperatures. Lownsbery (9) found that fewer *X. americanum* moved through a milk filter after storage in water at 34 C than at 20 C. He also found that nematodes moved through the filter as rapidly at 16 C as at 29 C. Barker et al. (1) reported that recovery of *X. americanum* from field soil by the Baermann funnel technique, which recovers active nematodes, was similar during 16 weeks of storage between 13 and 30 C without a host. Recovery was reduced at 2 and 36 C, and freezing at −15 C killed the nematodes. Temperature also affects reproduction and maturation of *Xiphinema* spp. (2, 6, 9, 10, 17), but this may not necessarily be related to virus acquisition and transmission.

Little has been reported on the effects of temperature on replication of TRSV and maintenance of titer in hosts. Hendrix (7) reported that a tobacco breeding line, which gave a resistant reaction to TRSV of local lesions and necrosis on inoculated leaves at 24 C, was systemically invaded at 35 C, indicating that TRSV replicates at 35 C.

The purposes of this study were to determine the effects of temperature on (i) acquisition and transmission of TRSV by *X. americanum,* (ii) maintenance of TRSV in roots of cucumber, (iii) replication of TRSV after mechanical inoculation of cucumber roots, and (iv) survival and movement of *X. americanum.*

MATERIALS AND METHODS.—*General.*—A watermelon isolate of TRSV (PV-125, ATCC) which is transmitted by *Xiphinema americanum* (4) was used in all tests. It is serologically identical to Gooding's NC-72 strain (5). The virus was purified (16) from infected cucumber (*Cucumis sativus* L. 'Model'), standardized at approximately 1.5 mg/ml in 0.05 M phosphate buffer (pH 7.0), and frozen. The suspension was thawed and diluted 1:100 when used to mechanically inoculate cucumber acquisition plants, or as described later for other tests.

Xiphinema americanum was reared in the greenhouse in a soil bed continuously cropped with Sudan grass [*Sorghum vulgare* Pers. var. *sudanense* (Piper) Hitchc. 'Piper'] (12).

Unless otherwise indicated, the medium for plant growth was fine river sand (11) autoclaved for 30 minutes. If nematodes were to be recovered from the sand, it was sifted through a 60-mesh sieve before use. Nutrients were provided by watering twice weekly with a soluble fertilizer solution.

Acquisition and transmission of TRSV by X. americanum.—Nematodes were handled for virus acquisition and transmission in the manner previously described (11). They were given a virus acquisition access period of 10 days, either at 28 C, or at various acquisition temperatures. After acquisition access, single nematodes were given 30 days of transmission access to cucumber bait plants in growth chambers or in water bath temperature tanks in the greenhouse at constant temperatures of 16, 22, 28, and 34 C. Virus symptoms were recorded when evident, and tops and roots of all bait plants were indexed (11) onto cowpea [*Vigna sinensis* (Torner) Savi 'Monarch' or 'Early Ramshorn'].

Maintenance of TRSV in roots of cucumber.—Cucumber seedlings in the cotyledonary stage were mechanically inoculated with TRSV. Following inoculation, the plants were held under fluorescent lights in the laboratory until a chlorotic mottle developed in the primary leaves. Roots of some plants were ground in buffer at this time and were indexed onto half-leaves of cowpea to determine the variability in titer of TRSV in roots of plants growing under the lights. The remainder of the plants were put into growth chambers set at 16, 22, 28, 30, 32, or 34 C. At intervals of 3, 7, and 10 days, roots were indexed and virus titer was

TABLE 1. Acquisition and transmission of tobacco ringspot virus to cucumber by *Xiphinema americanum* at various temperatures

Acquisition temperature (C)	Transmission temperature (C)	Total tests	Total plants	Transmission (%)[a]	
				Range	Average
16	16	2	40	...	0
	28	3	44	0-22	14
22	22	2	40	0-5	3
	28	2	44	10-33	25
28	16	4	85	...	0
	22	4	110	7-25	18
	28	8	194	25-80	46
	34	4	110	0-10	4
34	28	3	37	0-25	22
	34	1	28	...	7

[a]Single nematodes per cucumber bait plant.

determined by local lesion assay. A latin square design was used in indexing (13) so that each treatment was paired with all other treatments on adjacent half-leaves. This gave six replications of inoculum from each plant.

Mechanical inoculation with TRSV.—Cucumber seeds were planted in autoclaved coarse sand and placed under fluorescent lights in the laboratory until the seedlings were in the cotyledonary stage. The seedlings were removed from the sand and the roots were rinsed in distilled water and blotted. The roots were submerged in a 1:100 dilution of purified TRSV in a sterile petri dish and pricked several times with a No. 1 dental pulp-canal file. After inoculation, each cucumber seedling was transplanted into a 250-ml plastic cup of fine sand that had been moistened for several days. The cups with plants were set in chambers at 16, 22, 28, and 34 C. Roots of all plants were indexed onto half-leaves of cowpea 14 days later.

Survival of X. americanum.—Cucumber plants were grown in 100-ml beakers under fluorescent lights in the laboratory. Fourteen days after planting, five plants were set in each growth chamber at 16, 22, 28, and 34 C overnight, then 50 handpicked *X. americanum* were washed into the root zone of each plant. After 10-days of feeding access, the nematodes were recovered by screening (11) and the number of surviving nematodes was determined.

Movement of X. americanum.—Cardboard boxes 2.5 cm wide, 5 cm high, and 20 cm long were dipped in paraffin for waterproofing, then filled with fine sand. Two cucumber plants were grown at one end of some boxes under fluorescent lights in the laboratory. Other boxes were maintained in the same manner, but without plants. Twelve days after planting, the boxes were put into growth chambers at 16, 22, and 28 C overnight to adjust to the temperature, then 100 *X. americanum* were handpicked and washed into the sand in each box. Nematodes were added either at one end of the box (opposite end from the plants, if present) or in the middle of the box. After 10 days, which is the standard time used for virus acquisition access, the boxes were divided into segments, nematodes were recovered by screening, and the number of live nematodes recovered per segment was recorded. The boxes were divided into five segments as follows: When nematodes were added at the end of the box, the boxes were cut into segments of 2.5, 2.5, 5, 5, and 5 cm beginning at the end opposite where the nematodes were added. When the nematodes were added in the middle, the boxes were cut into segments of 2.5, 5, 5, 5, and 2.5 cm from end to end.

L-shaped boxes, 15-cm long on the outside of each arm, were used in a subsequent test. The nematodes were added to the 2.5-cm segment at the point of the "L" in boxes with two cucumber plants growing at the end of one arm of the "L", and in boxes without plants. After 10 days, nematodes were recovered from segments of 2.5, 5, and 5 cm on each arm beginning at the ends, and from the 2.5 cm square at the point of the box where the nematodes were added.

RESULTS.—*Acquisition and transmission of TRSV by X. americanum.*—Tobacco ringspot virus was acquired by *X. americanum* at 16, 22, 28, and 34 C and

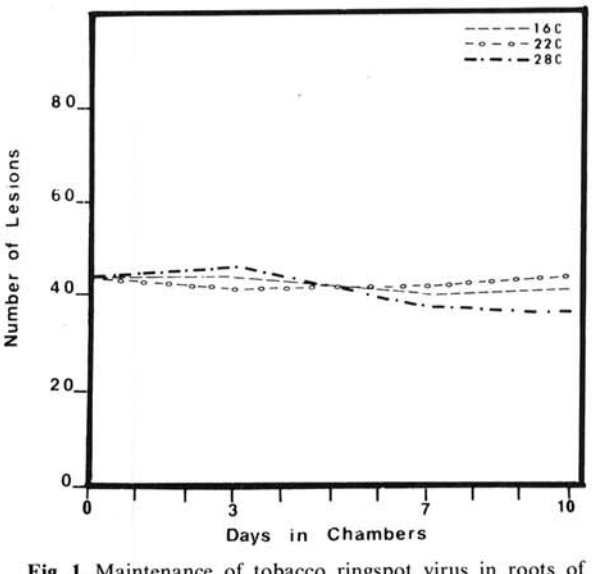

Fig. 1. Maintenance of tobacco ringspot virus in roots of cucumber held for 10 days after symptom development in growth chambers at 16, 22, and 28 C.

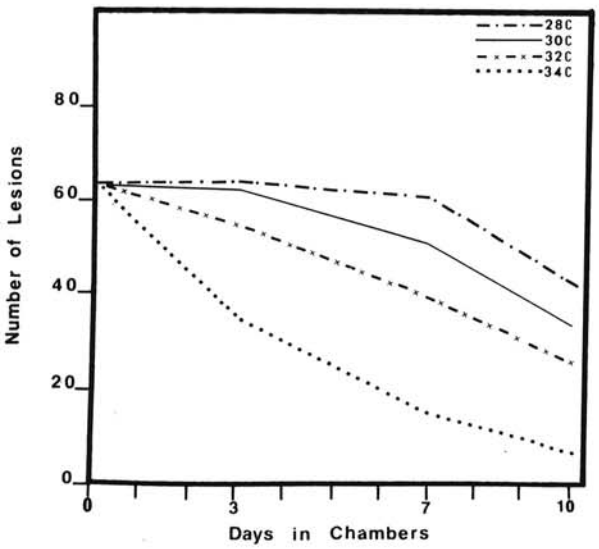

Fig. 2. Maintenance of tobacco ringspot virus in roots of cucumber held for 10 days after symptom development in growth chambers at 28, 30, 32, and 34 C.

TABLE 2. Survival of *Xiphinema americanum* during 10-days of feeding access to cucumber at various temperatures

Temperature (C)	Nematodes recovered (%)	
	Test 1	Test 2
16	40[a]	51
22	49	52
28	39	40
34	6	4

[a]Numbers are percent of nematodes recovered from five plants with 100 nematodes added per plant.

was transmitted at all temperatures except 16 C (Table 1). The optimum temperature for acquisition and transmission was 28 C. When nematodes acquired TRSV at 16, 22, or 34 C, a higher percentage transmitted at 28 C than at the acquisition temperature (Table 1). Rates of transmission were decreased more than rates of acquisition at temperatures above and below the optimum.

Maintenance of TRSV in cucumber roots.—The titer of TRSV maintained in the roots of infected cucumber plants did not differ greatly at 16, 22, and 28 C during a 10-day period (Fig. 1). Above 28 C, titer decreased steadily during the 10-day period, particularly at 34 C (Fig. 2).

Replication of TRSV after mechanical inoculation of roots.—Virus was recovered from roots of some cucumber plants at all temperatures 2 weeks after mechanical inoculation of roots. Ten percent of plants at 16 C were infected, 30% at 22 C, 23% at 28 C, and 26% at 34 C.

Survival of X. americanum.—In tests of acquisition and transmission of TRSV by *X. americanum* (see above) nematode recovery from cucumber acquisition plants usually averaged 40-50% of the number added when the acquisition temperature was 16, 22, or 28 C. However, recovery was very poor after acquisition access at 34 C. Two tests were designed specifically to determine survival of *X. americanum* during 10-days of feeding access to healthy cucumber. Percent surviving nematodes recovered ranged from 39-52% at 16, 22, and 28 C, but averaged only 5% at 34 C (Table 2).

Movement of X. americanum.—At 16 C, some nematodes added at the end of boxes 20 cm long had moved at least 15 cm after 10 days (Table 3). Nematodes added in the middle of the boxes moved in either direction throughout the boxes. Presence of plants did not seem to attract the nematodes. After 10 days at 22 and 28 C, some nematodes added at the end of the boxes had moved at

TABLE 3. Movement of *Xiphinema americanum* in fine river sand with and without cucumber host plants during a 10-day period

Temperature (C)	Host present	Nematodes added[b]	Percent of nematodes recovered from segments[a]				
			1	2	3	4	5
16	yes	End of box	69[c]	25	6	0	0
	no	End of box	67	20	11	2	0
	yes	Middle of box	11	20	33	29	7
	no	Middle of box	7	18	48	23	4
22	yes	End of box	39	28	24	7	2
	no	End of box	39	36	17	6	2
	yes	Middle of box	2	15	43	33	7
	no	Middle of box	3	23	32	32	10
28	yes	End of box	9	30	48	11	2
	no	End of box	22	16	47	12	3
	yes	Middle of box	0	3	38	54	5
	no	Middle of box	3	5	32	55	5

[a]Boxes (2.5 cm wide, 5 cm high, and 20 cm long) in which nematodes were added at the end were cut into five segments of 5, 5, 5, 2.5 and 2.5 cm which are segments 1-5 respectively. Segment 1 is from the end of the box where the nematodes were added. Boxes in which nematodes were added in the middle of the box were cut into five segments of 2.5, 5, 5, 5, and 2.5 cm which are segments 1-5 respectively. Segment 3 includes the area where nematodes were added. Plants, when present, were in segment 5.

[b]One-hundred handpicked *X. americanum* washed into the sand.

[c]Numbers are percent of recovered nematodes and are the average of four replications.

least 17.5 cm (Table 3). Larger numbers of nematodes moved greater distances from where they were added as the temperature increased (Table 3). At 22 C, nematodes moved throughout the boxes with or without plants; i.e., some of the nematodes added in the middle of the boxes moved away from the plants. When nematodes were added in the middle of boxes at 28 C, almost all nematodes moved toward the plants (Table 3), but in two of four replications almost all of the nematodes added in the middle of boxes without plants also moved in one direction.

In subsequent tests using L-shaped boxes, there was definite attraction of nematodes toward plants at 28 C (Fig. 3), some attraction at 22 C, and no attraction at 16 C.

DISCUSSION.—Several factors could be involved in the effect of temperature on acquisition and transmission of TRSV by *X. americanum*. These are: availability of virus to the nematodes in roots of acquisition source plants, ability of virus to replicate in roots of bait plants at the different temperatures following transmission, survival of nematodes, and movement and feeding of nematodes at the various temperatures.

Poor survival of *X. americanum* seems to be the sole reason for low levels of transmission of TRSV at 34 C. Nematodes that do survive this temperature can acquire and transmit the virus. Although virus titer in the roots of cucumber acquisition plants decreased steadily during a 10-day period at 34 C, sufficient titer is maintained for nematodes to acquire the virus. TRSV seemed to replicate well in cucumber roots at 34 C following nematode or mechanical transmission. Therefore, if nematodes introduce the virus, plants should become infected. The work of Hendrix (7) showed that TRSV replicated in tobacco at 35 C.

At temperatures below the optimum of 28 C, decreased acquisition and transmission of TRSV by *X. americanum* seems to involve the interaction between the nematodes and cucumber. Most of the nematodes moved toward cucumber plants at 28 C, less were attracted at 22, and there was no indication of attraction at 16 C. Schmitt (15) showed that *X. americanum* was attracted upward to roots of lilac in two soil types, but that they moved deeper into the soil if plants were absent.

It seems likely that the lower rate of transmission of TRSV by *X. americanum* at 22 C, and the lack of transmission at 16 C, is related to attractiveness of cucumber as a feeding host at these temperatures. Lownsbery (8) reported that cucumber was a poor host for reproduction of *X. americanum* in experiments conducted at an average temperature of 21 C suggesting lack of feeding at this temperature. Although the distance which a majority of nematodes moved from the point where they were added into fine river sand decreased as the temperature decreased, many nematodes moved enough at all temperatures to suggest that they would ordinarily contact roots in the small containers in which acquisition and bait plants were grown in these tests. TRSV is available to the nematodes in approximately equal amounts in acquisition plants at 16, 22, and 28 C, yet rates of acquisition by the nematode are much less at 22 and 16 C. Lownsbery (9) found that *X. americanum* was equally active in water at 16 and 29 C.

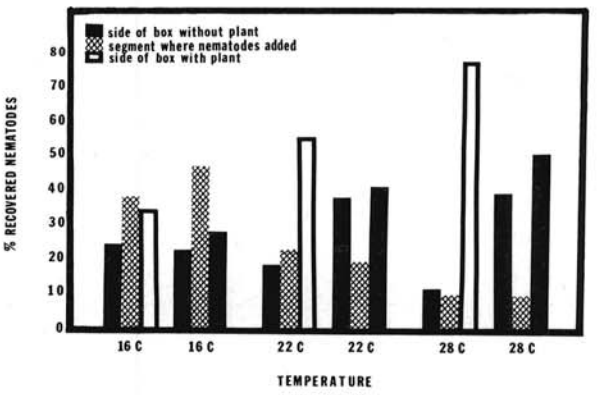

Fig. 3. Movement of *Xiphinema americanum* at various temperatures in fine river sand in L-shaped cardboard boxes with and without cucumber plants.

The lack of difference in amount of mechanical transmission to roots at 22 and 28 C and infection of several cucumber plants at 16 C also suggest that temperature effects are mostly on nematode feeding at these temperatures. *X. americanum* failed to transmit TRSV in several tests at 16 C. The percent mechanical transmission at 16 C was lower than at other temperatures; however, since some plants became infected, TRSV can replicate at this temperature.

The acquisition and transmission data suggest a different type of feeding by *X. americanum* for acquisition than for transmission. This might also be explained by greater attraction of nematodes to TRSV-infected cucumber than to noninfected cucumber. Inability to observe feeding of *X. americanum* makes it very difficult to determine the basis for these differences.

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Pages 135 and 137, the running heads should read TRSV instead of TMV.