

Maize Streak Virus: Effect of Temperature on Vector and Virus

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Appreciation is extended to Joan M. Snapp, Doris L. Hahn, and Denise Knott for valuable technical assistance in the project.

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This is the second of a series of articles on maize streak and its causal agent.

Accepted for publication 7 June 1984.

ABSTRACT

Damsteegt, V. D. 1984. Maize streak virus: Effect of temperature on vector and virus. *Phytopathology* 74:1317-1319.

The role of temperature in the biology of the leafhopper, *Cicadulina mbila*, and on the transmission and storage of maize streak virus, was investigated. Constant temperatures between 10 and 25 C and diurnally alternating temperatures from 0 to 27 C were used to study oviposition rates, nymphal development, mortality, and virus incubation. Optimal temperatures were either a constant 25 C or alternating 27 C day/21 C night. No oviposition occurred at <10 C. Insect mortality and adult longevity increased as the mean temperature decreased from 25 to 10 C. The time from inoculation with maize streak virus to symptom development in

maize was inversely related to increase in temperature. Transmission occurred in all alternating temperature regimes although maize seedlings did not show symptoms when maintained at <10 C because of poor plant growth. Partially purified preparations suspended in 0.01 M phosphate buffer were not infective after storage for 30 days at 4 C or after 370 days at -20 C. Cryogenic temperatures between -125 and -180 C provided excellent short- and long-term storage of MSV in infected leaves or in a partially purified preparation. No loss in infectivity was detected after 2,410 days of cryogenic storage.

Additional key words: exotic pathogens.

Temperature is one of the principal environmental parameters influencing the plant disease process. In virus-induced diseases with obligate arthropod vectors, temperature may affect the vector, the virus, the transmission process, the host, and/or the disease process. Temperature and moisture requirements have geographically restricted maize streak virus (MSV), which is transmitted by six species of leafhopper (*Cicadulina* spp.), to tropical and subtropical areas of the eastern hemisphere (12).

Studies on the effect of constant temperature on fecundity, length of stadia, and mortality of *Cicadulina* spp. have been published by Ammar (1), Rose (11), and Van Rensburg (15). Constant temperatures <15 C inhibited development of *Cicadulina mbila* Naude (15). Constant temperature of 30 C allowed life-cycle completion of *C. mbila* (11), but 31 C prevented its maturation (15) indicating a critical maximum temperature. In his experiments, Van Rensburg calculated that the optimum temperature for development of *C. mbila* was 25 C (15). Changing temperatures occur in field environments; Ammar found that fecundity and development of *C. bipunctella zae* China was enhanced by fluctuating temperatures between 17 and 33 C and that temperatures between 6 and 24 C during the winter months allowed life cycle completion (1).

Populations of *C. mbila* have been reported to withstand freezing temperatures for short periods, but no definitive studies have been conducted (12,15). Salt (13) indicates that leafhopper mortality rates are lower with sudden, rapid drops in temperatures than with gradual cooling found in nature. Decker and Cunningham (6) found that mean temperatures of 4-5 C increased survival time but prevented reproduction of *Empoasca fabae* (Harris). Subfreezing temperatures of -4 C for 48 hr killed all adult forms.

Determination of the potential threat of MSV to maize crops in the United States requires knowledge of the survival and reproductive potential of the vector and transmissibility of the virus under the temperature conditions found in the major maize-producing areas of the United States. Because of the research restrictions that are required when working with exotic pathogens, considerable effort was directed toward maintenance of "type cultures" for comparisons of experimental results over time.

Plant virologists routinely use cryogenic storage, lyophilization, air-drying over CaCl₂, or other techniques to maintain sap-transmitted virus cultures (9). Short- and long-term storage is possible with some persistently transmitted viruses by haemolymph injections of stored, infectious plant extracts (7,9,10). Rochow et al (10) described several methods for storage of barley yellow dwarf virus that maintained high levels of infection after 48 mo. Damsteegt et al (5) reported cryogenically storing maize streak virus for several months as partially purified preparations, and Timian (14) reported cryogenically storing wheat striate mosaic virus indefinitely. Kimura and Black (8) found that wound tumor extracts diluted to 10⁻² in histidine-MgCl₂ solution could be quick frozen and maintained at -80 C for at least 9 mo with no detectable loss in infectivity to vector cell monolayers.

This paper describes a simple method of preserving "type cultures" of MSV, an obligately leafhopper-vectored plant virus, and reports some effects of temperature on the biology of the virus and vector.

MATERIALS AND METHODS

The MSV isolate and vector, *C. mbila*, were hand-carried from Potchefstroom, S. Africa in 1974 (3,16) and have been studied in our containment facilities (3,4). Colonies of *C. mbila* were reared on DeKalb XL 45 or XL 43 maize plants in 30 × 30 × 61-cm Plexiglas™ cages at 26-28 C under natural daylight with supplemental fluorescent illumination to provide a 16-hr light/8-hr dark cycle.

Alternating temperature studies. Studies were conducted in controlled-temperature cabinets to measure the effect of diurnally alternating temperatures ranging from 0 to 27 C on insect

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mortality, virus transmission, and incubation period in the host. Five temperature regimes, 27 C day/21 C night (27/21 C), 21/15 C, 15/10 C, 10/5 C, and 5/0 C were utilized. Treatments consisted of four DeKalb XL 45 seedlings each caged with five viruliferous adult *C. mbila* for 5 days before removal by aspiration. The seedlings remained in the temperature cabinets for 18 days before transfer to 23 C for an additional 17 days of observation. Controls were caged for the same time period with nonviruliferous *C. mbila*. Each experiment was repeated three to five times.

Concurrent with the *C. mbila*/MSV experiments, a temperate vector/virus combination [*Graminella nigrifrons* (Forbes) and maize chlorotic dwarf virus (MCDV)] was tested to compare subtropical and temperate vector systems. Five MCDV-infective *G. nigrifrons* were caged on each of four maize seedlings for 5 days then removed by aspiration. Seedlings remained at the test temperatures for 18 days before transfer to 23 C for an additional 17 days. Selected temperature regimes were repeated three times.

Constant-temperature studies. The effects of constant temperature on insect mortality, fecundity, and nymphal development were compared to the effects of diurnally alternating temperatures. The four treatment temperatures (10, 15, 20, and 25 C) were maintained for 45 days and repeated three times. Ten adult, nonviruliferous males and 10 females were caged at each temperature for the duration of the test or until death. The number of surviving adults, oviposition sites, number of offspring, and nymphal development were observed.

Virus storage studies. Storage studies were conducted with partially purified MSV (2) and/or infected leaf pieces. Partially purified virus pellets from high-speed centrifugation were resuspended in 0.01 M phosphate buffer (pH 7.4) and stored in screw-cap vials at 4, -20, and -125 to -180 C (gaseous phase of liquid nitrogen refrigerator). Samples were withdrawn at intervals from 1 hr to 370 days, thawed at 40 C, and used immediately in membrane feeding tests.

Frozen infected leaves were removed at intervals from 30 to 2,410 days. Recovery assays of samples stored <30 days were accomplished by macerating thawed leaf pieces in 0.01 M phosphate buffer and subjecting the mixtures to low-speed centrifugation (6,000 g for 10 min). The supernatant was mixed with granular sucrose to produce a 5% sucrose mixture which was placed on stretched parafilm membranes. Nonviruliferous *C. mbila* were fed on the inoculum through the membranes for 24 hr and then transferred singly to healthy seedlings. All recovery tests beyond 30 days of storage were done by giving nonviruliferous *C. mbila* a 24-hr acquisition feeding on thawed leaf pieces before transferring them singly to maize seedlings for a 72-hr inoculation feeding. Controls for each storage assay consisted of feeding nonviruliferous *C. mbila* on fresh MSV-infected corn for 24 hr,

transferring them singly to test seedlings for 72 hr, and then removing them by aspiration.

RESULTS

Transmission of MSV occurred in all temperature regimes. The shortest incubation period for MSV in maize seedlings occurred at 27/21 C and the longest incubation at 5/0 C (Table 1). Test plants grew very poorly and became chlorotic in the 10/5 C and 5/0 C regimes. No symptoms were observed in the 5/0 C regime until surviving plants began to rejuvenate at 23 C. This indicated that transmission had occurred earlier.

Insect mortality was greater with *C. mbila* than with *G. nigrifrons* in all temperature regimes. Both species suffered much higher mortality as the temperature approached freezing (Table 1). There was no difference in survival between viruliferous and nonviruliferous adult *C. mbila*.

In one 5/0 C experimental trial the temperature cabinet controls malfunctioned and the temperature dropped to -5 C for 8 hr. All seedlings froze to the ground, ice formed on the tray, but one of 20 *C. mbila* and 11 of 20 *G. nigrifrons* survived on the frozen stumps. A second 8-hr period at -5 C the following day killed all remaining insects.

Constant temperatures of 10 and 15 C extended the longevity of *C. mbila* (9/20 and 3/20 original adults, respectively) but inhibited reproduction and development (none at 10 C and few nymphs at 15 C). No original adults survived 45 days at 20 or 25 C. More than twice as many eggs were produced and nymphs hatched at 25 than at 20 C; all nymphal stages as well as second generation adults were present in 25 C chamber by 45th day. The 25 C temperature was optimal in these studies.

No transmission was obtained from partially purified virus preparations stored at 4 C for 30 days (Table 2). Following the initial negative results at 30 days, all remaining samples stored at 4 C were tested immediately with five or more *C. mbila* per seedling with no transmission.

Transmission was obtained with virus stored at -20 C for up to 120 days. The transmission percentage had dropped to 10% at 218 days and no transmission occurred after 370 days or 2,115 days (Table 2).

High levels of transmission were obtained with partially purified preparations stored in the gaseous phase of a liquid nitrogen

TABLE 1. Effect of alternating diurnal temperatures on *Cicadulina mbila* and *Graminella nigrifrons* survival and maize streak virus (MSV) and maize chlorotic dwarf virus (MCDV) incubation periods^a

Treatment temperature ^b (C)	Insect survival (%)			Virus incubation (days)	
	<i>C. mbila</i>		<i>G. nigrifrons</i>	MSV	MCDV
	Viru- liferous	Nonviru- liferous			
27/21	76	79	... ^c	3.7	5 ^d
21/16	76	81	...	4.7	...
16/10	73	77	92	7.5	21
10/5	61	61	85	10 ^e	21
5/0	30	42	68	20.5 ^e	28

^a *C. mbila* transmits MSV and *G. nigrifrons* transmits MCDV.

^b Numerator is day temperature (16 hr) and denominator is night temperature (8 hr).

^c No test conducted.

^d Normal incubation of MCDV at 25 C is 5 days.

^e All plants turned yellow when kept at 5/0 C for 18 days. After 3 days at 23 C, those that survived turned green and symptoms were visible. Many plants in the 10/5 C regime also had become chlorotic by the 18th day of the test.

TABLE 2. Infectivity of maize streak virus following different storage treatments assayed by feeding nonviruliferous *Cicadulina mbila* directly on leaf pieces or on a buffered extract through stretched Parafilm membranes

Treatment ^a	Length of storage	Storage conditions ^b		
		4 C	-20 C	Gaseous phase LN ₂ (-125 to -180 C)
Partially purified prep	0	6/9 ^c	6/9	6/9
	1 hr	4/6	... ^d	9/10
	30 days	0/10	7/10	...
	116 days	...	5/10	7/10
	218 days	...	1/10	...
	370 days	...	0/10	6/10
MSV-infected leaves	0	6/7
	30 days	9/10
	742 days	5/6
	1,061 days	17/21
	1,457 days	17/20
	1,850 days	19/24
	2,115 days	...	0/10	19/26
	2,410 days	10/14

^a Partially purified pellets resuspended in 0.01 M PB, pH 7.4, or infected leaf pieces.

^b Storage conditions: Partially purified resuspended centrifugation pellets at 4 C, -20 C, and gaseous phase of liquid nitrogen (LN₂) refrigerator; infected leaves at -20 and gaseous phase of an LN₂ refrigerator.

^c Number infected/number inoculated.

^d No test conducted.

refrigerator from 1 hr to 370 days. No difference in degree and type of infectivity was detected over the storage period (Table 2).

Samples of cryogenically frozen MSV infected leaves were withdrawn from the liquid nitrogen refrigerator periodically from 30 to 2,410 days after storage. Adult *C. mbila* given a 24-hr acquisition feed on the thawed leaf pieces transmitted virus at levels equal to the originally stored material. Thawed leaf pieces began to ferment at room temperature by 24 hr and were no longer palatable to leafhoppers by 48 hr.

DISCUSSION

Temperature plays an important role in ecology of insect-transmitted plant viruses. Constant cool temperatures (15 C) favor increased longevity of adult *C. mbila* but retard oviposition and nymphal development (11,15). Constant temperatures, however, do not exist in the field. Results of studies by Ammar (1) with *C. bipunctella zae* indicated that a much wider range of alternating temperatures promoted fecundity and development. In our studies, we found that alternating temperatures of 16 C day/10 C night, 21/16 C, and 27/21 C had no effect on mortality or transmissibility of MSV, but temperatures of 10/5 C and 5/0 C adversely affected survival.

Cicadulina spp. do not possess a physiological mechanism for overwintering in cold climates. As the temperatures approached freezing, the mortality of the adults rose more sharply than it did with adults of the temperate leafhopper, *G. nigrifrons*. All of our temperature studies were conducted in a time span of three consecutive generations of *C. mbila*; therefore, we could not determine the adaptation of the population of *C. mbila* to gradually cooling temperatures. Our leafhoppers were reared at 25–30 C before placement in the test temperature regimes.

The absence or presence of contact moisture at low temperatures affects mortality of *C. mbila* (15). We maintained leafhoppers in cages where the relative humidity often exceeded 75% and condensation formed on the cage walls. The high humidity may have accounted for the high mortality in the 5/0 C regimes and in the subzero test.

Decreasing temperatures from 27/21 to 5/0 C had no effect on transmissibility of MSV with 100% infection in all surviving plants although the time from inoculation to symptom appearance increased in inverse proportion to decreasing temperature. Maize grew poorly at temperatures <10 C and leaves on the resulting plants were chlorotic. Normal growth resumed following transfer of the plants to 23 C and typical streak symptoms appeared in all surviving plants. This indicates that leafhoppers had transmitted the virus in the first 5 days of the test and that the virus had become established in the seedlings growing under cool conditions.

Storage of MSV at cryogenic temperature (–125 to –180 C) provided an excellent environment for preserving the infectivity of the isolate. Reisolation and establishment of the virus in new plants following its acquisition from thawed leaves by *C. mbila* on at least eight separate occasions during 8 yr with no change in length of time for symptom appearance, vector efficiency, or symptom expression indicates the stability of MSV in cryogenic storage.

Kimura and Black (8) utilized cryo-preservation of wound tumor virus extract for obtaining a standard inoculum for insect cell

monolayers. They were able to develop a rapid and reliable infectivity assay for wound tumor virus with cell monolayers which maintained a constant infectivity for at least 9 mo (8). Our technique for maintaining a standard virus type by cryogenic storage and reestablishment in plants by feeding *C. mbila* on thawed leaf pieces is comparable to their standard inoculum. However, we are transmitting an obligately insect-borne virus from plant to plant instead of from insect tissue to insect tissue. This technique has been successful with MSV and wheat striate mosaic virus (14). However, repeated attempts to recover MCDV with *G. nigrifrons* following storage in ultra-cold have been unsuccessful.

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