

# Transmission of an Eriophyid-borne Wheat Pathogen by *Aceria tulipae*

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## ABSTRACT

An investigation was made of the transmission of a disease agent, similar to the wheat spot mosaic virus of Slykhuis, by its eriophyid mite vector, *Aceria tulipae*. This agent is referred to as the wheat spot chlorosis pathogen (WSCP). All stages of the mite, first and second instars and adults transmitted WSCP when the pathogen was acquired by mites during nymphal stages. But when adults were given 48-hr access to excised infected leaves, none of 240 mites transmitted WSCP to test plants. Comparable groups of nymphs transmitted WSCP to 23 of 24 test plants. In other tests, nymphs acquired and transmitted WSCP when given 1-hr access to ex-

cised infected leaves. None of 175 mites which developed from eggs taken from diseased wheat transmitted WSCP, indicating that the pathogen is not transovarially passed. Second instar mites continued to transmit WSCP after a molt with no reduction in transmission rate when compared to nonmolted second instars. The rate of transmission of the pathogen by single adults maintained on an artificial medium declined from 30.7% for mites at 0 days to 7.3% after 8 days. No mites transmitted after 10 days on the medium. *Phytopathology* 60: 1616-1618.

An eriophyid-borne pathogen transmitted by the wheat curl mite, *Aceria tulipae* (Keifer), was recently recovered from diseased wheat and corn in Ohio (4). The host range and symptomatology of the pathogen are similar to that previously reported for the mite-borne wheat spot mosaic virus (WSpMV) (9). The pathogen has not been purified or isolated. Until additional properties such as morphology and chemical composition are ascertained, its relationship to WSpMV and the viral nature of the pathogen cannot be established. The causal agent has been designated as the wheat spot chlorosis pathogen (WSCP) until more completely characterized.

The pathogen may have unique characteristics unlike any previously reported. Electron microscopy of ultrathin sections of diseased wheat, barley, and corn have revealed double-membrane-bound ovoid bodies (0.1-0.2  $\mu$ ) in the cytoplasm of parenchyma, phloem, and epidermal cells (1). The internal components of these bodies consist of dispersed fibrils. No ribosomes characteristic of bacteria, mycoplasma, or the psittacosis group of organisms or an electron-dense nucleoid region characteristic of most viruses have been detected. It is not known whether these bodies are the pathogen or a novel entity resulting from the disease.

This paper will detail the transmission of WSCP by its vector, *A. tulipae*. From vector-pathogen relationship, an interpretation of the mode of transmission is presented. A preliminary report has been published (3).

**MATERIALS AND METHODS.**—The wheat curl mite used in these studies was originally obtained from field corn in Wayne County, Ohio. A colony of pathogen-free mites was established by infesting healthy wheat with mite eggs. Mites were reared on Cheyenne wheat in a Sherer<sup>12</sup> Model CEL 255-6 growth chamber maintained at 24 C with continuous light. In all experiments, mites were manipulated with a dowel-mounted hair.

The WSCP isolate used in this study was originally obtained from volunteer wheat. The pathogen was

maintained in Oh28 inbred corn in a growth chamber set at 21 C with continuous light. Plants in the two- to three-leaf stage were inoculated with infectious mites for a 25-hr inoculation period terminated by fumigation with dichlorvos. After 7 to 14 days, plants showing symptoms were infested with WSCP-free mites and placed in the growth chamber. This system allowed us to maintain both the pathogen and infectious mites.

Test plants were Redcoat wheat or Oh28 inbred corn. Plants in the two- to three-leaf stage were used in transmission tests. Mites were given specific inoculation access periods on test plants before fumigation with dichlorvos. Fumigation usually destroyed all mites on corn test plants; however, a second fumigation of wheat plants was often needed because of rolled leaves caused by mite feeding. Test plants were placed in a growth chamber or greenhouse for 2 weeks and observed for symptom development. Only 2 of over 500 noninfested control plants (Oh28 corn) placed with test plants showed symptoms of WSCP.

When mites were fed on excised leaf pieces, the following technique was used. A mixture of nine parts plaster of paris, one part activated charcoal, and water was layered (1.5 cm) on the bottom of a glass petri dish. When wet, a relative humidity of 95% or greater was established within the covered petri dish. We found that high humidity is essential to survival of eriophyid mites feeding on exposed leaf pieces. With this system, we attained excellent mite survival when fresh leaves were provided every 3 to 4 days.

**RESULTS.**—*Transmission efficiency by single mites.*—From February through September of 1969, several hundred single adult mites reared on infected corn were periodically transferred to corn test plants to determine individual transmission efficiency. During the spring and early summer, transmission was consistently near 50% but dropped to a low of <1% in late summer. In one test, when various mite stages were compared for transmission efficiency, 15/25 of adults,

8/25 of second instars, and 6/25 of first instars transmitted the pathogen.

*Acquisition and transmission of WSCP by mites.*—The following experiment was conducted to determine mite stages which can acquire and transmit WSCP, and to determine the min time for acquisition. Adult and second instar mites were placed on excised infected corn, wheat, or barley leaves in petri dishes. Mites were allowed 48-hr acquisition periods before transfer at the rate of 10/plant to eight corn test plants for each treatment. Mites were then given a 96-hr inoculation period. None of 240 adults acquired and transmitted the pathogen, while nymphs transmitted WSCP to 23 of 24 test plants.

First and second instar mites were given 1-, 2-, 4-, and 8-hr acquisition periods on excised infected barley or corn leaves in a petri dish before being transferred singly to corn test plants for a 96-hr inoculation period. In three experiments, a total of 50 mites was tested at each period. Mites transmitted the pathogen to 3, 0, 4, and 6 plants with 1-, 2-, 4-, and 8-hr acquisition periods, respectively.

*Transovarial passage of WSCP in mite.*—None of the eriophyid-borne pathogens are known to pass from adults to their progeny (10). The possibility of transovarial passage of WSCP was investigated. Adult mites and eggs were taken from infected wheat and transferred at the rate of five eggs or five adults/plant to wheat test plants for 48-hr inoculation periods. Evidence that eggs hatched and resulting mites fed was determined by inward rolling of leaves of test plants. Adults transmitted the pathogen to 34 of 35 test plants, while mites hatching from eggs transmitted to 0 of 35.

*Transmission of WSCP by molting mites.*—The following experiment was conducted to determine if WSCP is retained by molting mites. Eriophyid mites which are molting can be distinguished easily from non-molting individuals. The molting mite is quiescent and often appears translucent anteriorly and posteriorly. When lifted from the plant surface, the legs remain rigid anteriorly in contrast to the waving motion of legs of nonmolting individuals. To test for retention of WSCP by molted *A. tulipae*, second instar and molting second instar mites were removed from infected corn and transferred, five/plant, to corn test plants for a 72-hr inoculation period. In three experiments, molted mites transmitted the pathogen to 56/74 test plants, while nonmolted second instars transmitted to 58/74 test plants.

*Persistence of WSCP in the mite.*—The longevity of WSCP in the mite was determined while mites were maintained on an artificial medium. The medium was prepared by mixing 5 g potato-dextrose agar, 5 g of charcoal, and 40 ml of water and boiling the mixture. Ten ml of a 5% aqueous solution of Polymyxin sulfate was added to the medium after it had cooled and before it was poured into 3-cm watch glasses. The charcoal imparts a black color to the medium, which facilitates visibility of the mite and creates a texture on the agar surface which allows mites to move about freely. Adult mites were removed from infected corn and placed on

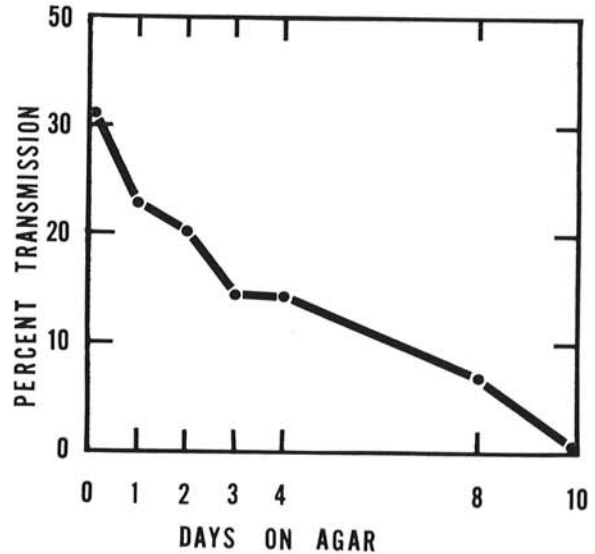


Fig. 1. Retention of wheat spot chlorosis pathogen by single *Aceria tulipae* maintained on an agar medium.

the agar medium. Mites were maintained on agar until removed at specific intervals and placed singly on corn test plants. As a check, mites were taken from infected corn and placed directly on test plants. From 25 to 75 mites were tested at each interval. Mites retained the pathogen for 8 days while on agar, but none of 25 mites transmitted after 10 days. There was a decline in transmission of the pathogen by mites, with an increase in time after removal from the infected source plant (Fig. 1).

*DISCUSSION.*—The biological relationships between *A. tulipae* and WSCP are similar to relationships reported for *A. tulipae* and wheat streak mosaic virus (WSMV) and WSpMV (Table 1). It is tempting to suggest from these empirical relationships that the underlying mode of transmission for these pathogens is similar.

WSMV and WSpMV are retained by *A. tulipae* after a molt. This is also true of *A. tulipae* in the transmission of WSCP. Retention of viruses by molted aphids is considered a criterion for the well established circulative relationships in aphids. Arthropods lose the cuticula

TABLE 1. Comparison of vector relationships of *Aceria tulipae* between wheat streak mosaic virus (WSMV), wheat spot mosaic virus (WSpMV), and the wheat spot chlorosis pathogen (WSCP)<sup>a</sup>

	Pathogen		
	WSMV	WSpMV	WSCP
Acquisition and transmission by adults	—	No data	—
Retention after a molt	+	+	+
Persistence > 1 week	+	+	+
Transovarial passage	—	—	—

<sup>a</sup> Results pertaining to WSMV are in Slykhuis (8); Del Rosario (2); and Orlob (5). Information for WSpMV is from Slykhuis (9, 10, 11).

lar lining and contents of the fore- and hindgut during a molt. Presumably only the contents of the midgut would remain. There has been no evidence presented to indicate that either aphids or eriophyids can regurgitate the midgut contents. It would be premature, however, to assume that flow of materials in the digestive tract of aphids and eriophyids is similar; therefore, the retention of pathogens by molting eriophyids may not be indicative of an identical circulative relationship.

A circulative route, and therefore a more complex biological relationship, could account for such phenomena as vector specificity and the failure of adult *A. tulipae* to acquire and transmit WSCP and WSMV. Apparently ingestion of WSMV by two nonvector eriophyid species is not the barrier to transmission. Orlob (5) observed viruslike particles by electron microscopy in homogenates of *Abacarus hystrix* (Nalepa) and *Aculodes mckenziei* (Keifer) which had fed on WSMV-infected wheat. Perhaps electron microscopy of ultrathin sections of these species would reveal the location of ingested WSMV and provide a basis for vector specificity. High concn of WSMV particles have been observed in the mid- and hindgut of ultrathin sections of viruliferous *A. tulipae* (6, 12). The gut wall of adult *A. tulipae* is apparently not a barrier to passage of particles into the vector's parenchyma tissue (12). Vector specificity, both inter- and intraspecific, may be controlled by uptake of the pathogen by the salivary glands. This is the suggested site for specificity of the aphid-borne, circulative, barley yellow dwarf virus (7). Takahashi & Orlob (12) did not find virus particles in the salivary glands of viruliferous *A. tulipae*; however, their sample was small (five mites), and virus could have easily been overlooked, particularly if only a few particles are necessary for transmission.

No data have been presented to indicate that any eriophyid-borne pathogen multiplies in its vector. Our results with WSCP would suggest that the pathogen does not propagate in *A. tulipae*. When mites were removed from a source of the pathogen, transmission

by single mites was eliminated after a 10-day period, indicating the gradual depletion of the pathogen reservoir in the mite. Pathogens which propagate in their vectors usually exhibit a constant level of transmission by their vectors once removed from a source of the pathogen.

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