Detection of Wheat Streak Mosaic Virus Antigens in Vector Mites with Fluorescent Antibodies

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ABSTRACT

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A fluorescent antibody technique was used to detect wheat streak mosaic virus antigens in crude "homogenates" prepared from 10 mites (*Aceria tulipae*) that had been exposed to virus-infected plants. The virus antigens also were detected in the body fluid released through the anus of individual exposed mites when pressed gently with a fine glass rod.

Wheat streak mosaic virus (WSMV) is transmitted by the mite *Aceria tulipae* Keifer and persists in the vector for several days (6). Electron microscopic examination of sections of viruliferous mites showed large concentrations of flexuous rod-shaped virus particles in the alimentary canal (4, 7). The virus also was detected in the homogenates of vector mites through manual inoculation of wheat (*Triticum aestivum* L. 'Kent') leaves or by employing a serological precipitin-ring test (4). In both of these tests, 250 to 800 mites are needed and because of the small size of the vector (200×75 μm) such tests are time-consuming and cumbersome.

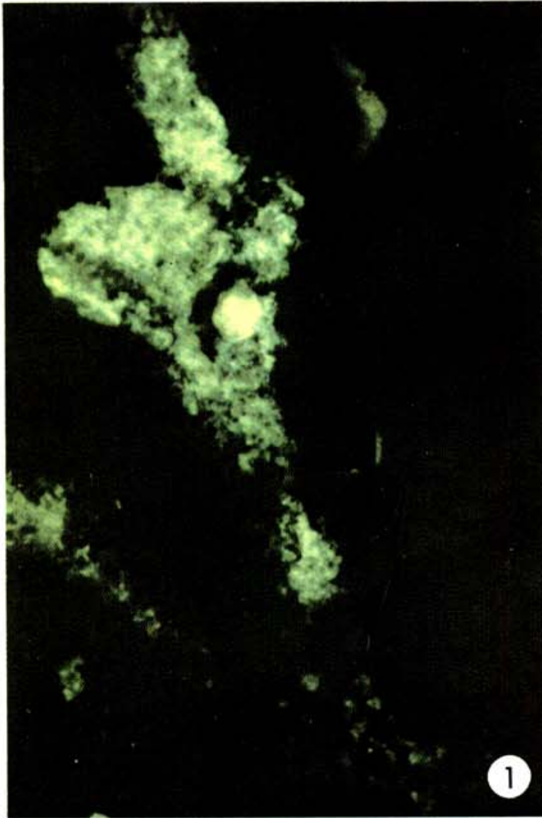
Ball (1) recently reported that a fluorescent antibody (FAB) technique can be used for detecting WSMV-antigens in sections of paraffin-embedded *A. tulipae*. Our results suggest yet another application of the FAB technique for the rapid detection of WSMV-antigens in mites that were exposed to virus infected plants.

Aceria tulipae, originally collected in Alberta, were maintained on wheat plants. Viruliferous mites were obtained by caging healthy mites on WSMV-infected wheat plants and allowing them to multiply for 2-3 weeks. Not all mites thus "exposed" to virus acquire it - those that do are referred to as "viruliferous". To prepare the crude homogenates, 10 young adult mites were mounted in a droplet of saline (0.85% NaCl) on a marked quartz slide smeared with a modified Haupt's adhesive (2). The saline was allowed to evaporate to a thin film and then the mites were mashed "en masse" using a fine glass rod (1-mm diameter). Such preparations were air dried for 30 minutes, heated on a hot plate (80 C) for 10 seconds, fixed in acetone for 10 minutes, and stained for 15 minutes with FAB at 0.5 dilution. The antiserum after conjugation with fluorescein isothiocyanate (FITC) had a precipitation end point of 1/80 as determined by ring interface test. Details of conjugating the antiserum or normal serum, staining procedure, and microscopy were as described earlier (5). In some experiments, mites were deposited individually on slides, and were gently pressed with the fine glass rod, thereby releasing some body fluid from the anus. The mites were then processed and stained with FAB as described above for the crude homogenate. Photographs were taken on a high-speed Ektachrome B film with 3 minutes exposure time.

In four experiments, nine spots, each with homogenates of 10 exposed mites and nine similarly prepared spots with homogenates of healthy mites were stained with FAB. All homogenates of exposed mites showed a brilliant yellow-green fluorescence (Fig. 1), but none of the homogenates of healthy mites showed such fluorescence (Fig. 2). Specificity of the staining was tested in two experiments by staining eight spots of homogenates of exposed mites with FITC-conjugated normal serum. None of the spots showed the yellow-green fluorescence and all were similar in appearance to those of healthy mites stained with FAB (Fig. 2). Furthermore, homogenates of *A. tulipae* infected with bromegrass mosaic virus (3) and stained with FITC-conjugated WSMV antiserum did not show the yellow-green fluorescence.

In an attempt to detect WSMV antigens in individual mites, 32 exposed and 36 healthy mites in three experiments were stained with FAB. Specific fluorescence (Fig. 3) was observed in the fluid released from the anus of 14 exposed mites. The remaining 18 exposed and 36 healthy mites did not show such fluorescence (Fig. 4). The percentage of exposed mites that were scored viruliferous by FAB technique is in agreement with that reported earlier (4) based on the presence of WSMV particles in thin sections of exposed mites.

Our results show that FAB technique is more efficient and rapid than previous methods in determining the number of viruliferous *A. tulipae* in a colony of mites exposed to the virus-infected plants. Also, it should be feasible now quickly to determine the numbers of



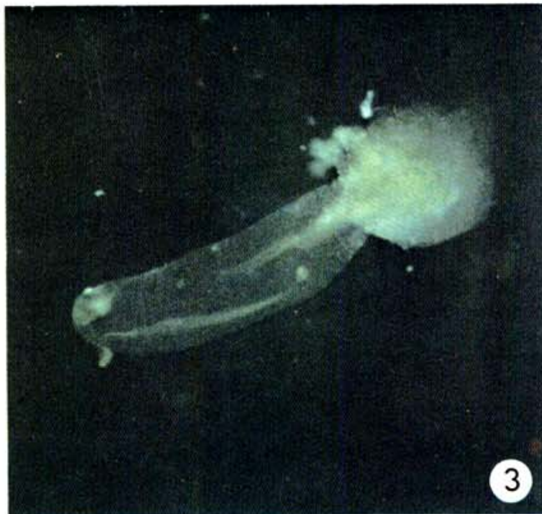


Fig. 1-4. Fluorescent antibody (FAB) detection of wheat streak mosaic virus (WSMV) antigen in vector mites *(Aceria tulipae)*. (Fig. 1-2) Fluorescent antibody (FAB)-treated homogenates of 10 mites; **1)** that had fed upon WSMV-infected wheat plants (note the bright yellow-green fluorescence); and **2)** that had fed upon healthy (nonWSMV-infected) wheat plants (only a dull yellow trace of fluorescence was observed). (Fig. 3-4) Demonstration of WSMV antigen in the body of a single *A. tulipae* (gently pressed to release fluid from the anus, then stained with FAB) **3)** that had fed upon WSMV-infected wheat plants; and **4)** that had fed upon healthy (nonWSMV-infected) wheat plants. Note the brighter yellow-green fluorescence associated with the mite that had access to WSMV antigen.

viruliferous *A. tulipae* in samples of wind-disseminated mites trapped over wheat fields. This information should be useful in predicting WSMV incidence in the crops.

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