60. Transmission of Sweet Clover Necrotic Mosaic Virus

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Sweet clover necrotic mosaic virus (SCNMV), a member of the dianthovirus group, possesses a bipartite genome and the following properties (Hiruki, 1986a): virions contain positive sense single-stranded genomic RNAs of approximate molecular weights (M_r) 1.35×10^6 (RNA-1) and 0.55×10^6 (RNA-2) and a coat protein of M_r 38×10^3 . The particles measure 33 nm in diameter and sediment at 126 S as a single component. It has a moderate host range, but causes characteristic necrosis, both local and systemic, often associated with severe stunting, which is a potential cause of reduction in crop yield. Research on modes of transmission is scarce. Insects have been suspected as natural aerial vectors (Hiruki, 1986b) but supportive evidence is lacking. This paper reports recent experimental data analysing the possible role of the western flower thrips *Frankliniella occidentalis* which is found in constant association with pollen grains in the florets of sweet clover plants both in the field and in the glasshouse.

Materials and methods. The SCNMV type strain 38 was originally isolated from sweet clover (*Melilotus officinalis*) in 1979 and has been maintained in the same host. For purification the virus was multiplied in *Phaseolus vulgaris* 'Red Kidney' and purified according to the method previously reported (Gould *et al.*, 1981).

All plants, unless stated otherwise, were grown in 12-cm-diameter pots containing an autoclaved mixture of loam, sand, and peat (1:1:1, v/v/v) in a glasshouse at $25 \pm 2^\circ\text{C}$.

Crude juice was obtained by grinding infected leaves with a mortar and pestle in the presence of 0.025 M phosphate buffer (Na_2HPO_4 - NaH_2PO_4), pH 7.0 at a fixed ratio. The extract was rubbed on leaves dusted with Carborundum. For local lesion assay, young seedlings of *Chenopodium quinoa* were used at growth stages of 4 to 8 uniform leaves.

Pollen grains and anthers were collected from selected flowers of infected and virus-free sweet clover (*M. alba*) plants maintained in 24-cm-diameter pots, by tripping the flower under a stereomicroscope.

Direct double sandwich enzyme-linked immunosorbent assay was used for SCNMV detection as described previously (Okuno *et al.*, 1983).

F. occidentalis, the western flower thrips, originally obtained from *M. alba* was reared on SCNMV-infected or on virus-free sweet clover in separate plant growth cabinets or separate glasshouse compartments. Adults and first and second instar nymphs were used either separately or as mixed populations.

Results. To test whether pollen grains released from anthers of sweet clover infected with SCNMV contain the virus, young healthy seedlings of *C. quinoa* were inoculated with aliquots of extracts from pollen samples or from thrips samples in three replicates. Other aliquots of both pollen and thrips

Table I. Detection of SCNMV antigen and infectivity in extracts of pollen and of western flower thrips that emerged from SCNMV-infected sweet clover in the glasshouse

Experiment	Pollen extract from ^a		Extract from ^a	
	Infected Sweet clover	Healthy Sweet clover	Viruliferous thrips	Nonviruliferous thrips
ELISA (A405)	>2.000	0.153	0.393	0.007
	>2.000	0.073	0.258	0.017
	>2.000	0.098	0.644	0.003
Local lesions ^b	55	0	1	0
on <i>C. quinoa</i>	29	0	8	0
	33	0	9	0

a. Pollen grains from 30 florets per pollen sample or 10 thrips nymphs per thrips sample were triturated in 0.025 M phosphate buffer, pH 7.0, and then the final volume of 100 μ l, made up with extraction buffer, was divided into two aliquots, one for ELISA and the other for local lesion assays. b. The figures represent average numbers of local lesions on 3 leaves.

samples were used for ELISA. Results of the experiments, show that pollen samples from virus infected sweet clover plants contained high concentrations of infective SCNMV, whereas those from healthy plants did not (Table I). The concentration and infectivity of SCNMV from extracts of viruliferous thrips remained relatively low.

Upon examination of thrips which had emerged from SCNMV-infected plants, pollen grains were found firmly attached to the surfaces of the thrips (results not shown).

Pollen collected from the flowers of sweet clover plants that had been experimentally inoculated and kept in the glasshouse for several months was washed three times. High concentrations of SCNMV were found in the first washing, significantly lower amounts in the second washing, and none in the third washing (Fig. 1). Similar results were obtained when the experiment was repeated with pollen samples collected from sweet clover plants that were infected naturally in the field (Fig. 2).

Thrips recovered from naturally infested sweet clover (*M. officinalis* and *M. alba*) both in the field and in the glasshouse were identified as *F. occidentalis*. In a series of surveys, the thrips were found in constant association with florets of sweet clover.

Twelve experiments were carried out using 3 to 4 young seedlings per treatment: a) thrips only, b) virus-containing pollen only, c) thrips and virus-containing pollen, and d) no treatment (control). Each treatment was done in a sealed Petri-dish at room temperature for 24 hours, following which the transplanted seedlings were kept in the glasshouse for 4–6 weeks prior to being tested for SCNMV using the ELISA technique. None of the experiments showed transmission of SCNMV by the thrips.

In another series of experiments both a mature, healthy sweet clover plant and a plant that had been mechanically inoculated with SCNMV were fully infested with thrips and then they were caged with 12 young seedlings of sweet clover test plants for a period of 7 weeks. Assays for SCNMV infection at the end of incubation showed that none of the test plants was infected with SCNMV.

Fig. 1. Detection of SCNMV by ELISA in pollen washing from sweet clover experimentally inoculated in the glasshouse. Pollen grains from 100 virus-infected florets were placed on a Millipore filter ($0.22 \mu\text{m}$) and washed using 1.2 ml extraction buffer (first washing) and then washing was repeated in the same way to obtain second and third washing. Pollen grains from 100 virus-free florets were subjected to the same treatment as controls. V, virus-containing pollen grains; H, virus-free pollen grains.

Fig. 2. Detection of SCNMV in pollen washing from naturally infected sweet clover in the field. Pollen grains from 20 florets of field samples 89/27 and 89/19 were washed using 0.8 ml extraction buffer at a time to obtain first or second washing as before. A comparable test was done with pollen grains from virus-free plants. VP, virus-containing pollen grains; HP, virus-free pollen grains.

In an additional experiment using 8 sweet clover plants with floral buds which were ready for blooming, no SCNMV transmission was detected after a 4-week exposure to a mixed population of thrips from a SCNMV-infected sweet clover plant.

Discussion. *F. occidentalis* is known as an efficient pollinator and several individuals often occur in each floret, and, in contrast to bees, they can penetrate

a flower bud before it opens (Levis, 1973). Thus, they are exposed to pollen for a longer time. In this investigation, up to 80 florets were found on a single floral stalk of an average sized, potted sweet clover plant in the glasshouse (data not shown). Since numerous floral stalks develop on a single sweet clover plant, the population level of *F. occidentalis* is quite high. During our observation, many thrips were found to be carriers of pollen grains which in turn carry virus particles. Pollen samples collected from virus-infected plants both in the field and in the glasshouse contained high titres of SCNMV antigen that is highly infectious (Figs. 1 and 2). However, the concentration of SCNMV detected in the extract of viruliferous thrips was not high enough to suggest replication of the virus in the thrips (Table I). Also, our results did not show any evidence of virus transmission by the thrips. Rather, our observations tended to show that sweet clover pollen grains are surface-contaminated with the virus in a similar manner to that reported previously (Francki and Miles, 1985; Hamilton *et al.*, 1977), and transmission of SCNMV to sweet clover plants is probably caused mechanically (Francki and Miles, 1985) when a significant amount of viral inoculum is released from the pollen grains of virus-infected plants. Therefore, the mode of transmission does not resemble that of tobacco streak virus, in which *Thrips tabaci* transmits pollen-borne virus (Sdoodee and Teakle, 1987). Further investigation is necessary to determine whether SCNMV is transmitted by thrips from floret to floret.

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