A chlorotic mosaic of fall hawkbit (Leontodon autumnalis)

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A short rigid rod-shaped virus with length classes of 140 nm and 160 nm was found to cause a chlorotic mosaic in 'fall hawkbit' (Leontodon autumnalis), a common weed found in potato-growing areas of eastern Canada. The 'fall hawkbit' virus (FHV) is serologically related to Hypochoeris mosaic virus (HMV), a recently described virus found in western Canada. In spite of other similarities between FHV and HMV there were some apparent differences in host range.

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Un virus court, rigide et a forme de bâtonnet de 140 à 160 mm de longueur est responsable d'une mosaïque chlorotique du liondent d'automne (Leontodon autumnalis), mauvaise herbe commune rencontree dans les regions de culture de pommes de terre de l'est du Canada. Le virus est serologiquement apparente au virus de la mosa'ique de l'hypochoeris, virus recemment decrit et repandu dans l'ouest du Canada. Malgré certaines autres similarités entre ces deux types de virus, ils affichent certaines differences dans la spécificité de leur hôte.

Introduction

The 'fall hawkbit' *(Leontondon autumnalis* L.) is a common weed in potato-growing areas of eastern Canada. In a survey for possible perennial weed hosts of potato viruses, this species was frequently observed showing a striking yellow mosaic. Since it had been reported from Europe that tobacco rattle virus (TRV) causes a chlorotic spotting in *Hieracium* L. (2), a genus closely related to *Leontodon,* it was initially speculated that TRV might be the causal agent of the disease in 'fall hawkbit'. Studies were therefore commenced to examine this possibility. However, during the course of this investigation a newly described virus named Hypochoeris mosaic virus (HMV) was reported to infect another close relative of *Leontodon, Hypochoeris radicata* L. (1) in western Canada. We here report that the chlorotic mosaic of 'fall hawkbit' is not caused by TRV but by a virus similar to HMV.

Materials and methods

Test plants were grown in a mixture of soil, peatmoss, and sand (2:1:1) in 10 cm clay pots. The virus was propagated in *Nicotiana tabacum* L. cv. Samsun, in a greenhouse maintained at 14-18°C. Infectivity assays were made on *Chenopodium amaranticolor* Costs & Reyn.

Leaf cell extracts were negatively stained with 2% ammonium molybdate, pH 7, using the leaf chopping method (5) and were examined with a Philips 201C electron microscope. The magnification was calibrated as before (5).

Serological testing was done with the SDS-agar diffusion method (4). Antisera to morphologically similar viruses were kindly supplied by Drs. R. Stace-Smith (HMV), H. Huttinga (five isolates of TRV), and L. Bos (Pea early browning virus).

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Results

The perennial weed, 'fall hawkbit', was commonly found on pasture lands and field borders. Naturally infected 'fall hawkbit' plants were observed in the spring (May to early June) when maximum daily temperatures were about 10-15°C. Often 5-20% of the plants in an area were symptomatic. The newly developing leaves showed chlorotic spotting (Fig. 1). This symptom became less apparent and disappeared when temperatures reached about 25"C but reappeared late in October when temperatures were cooler. Recovery of viral infectivity was invariably associated with the presence of this symptom: its disappearance in summer coincided with a loss of infectivity.

The virus from 'fall hawkbit' (FHV) was initially isolated by grinding the fresh leaf tissue in glycine-phosphate buffer (0.05M glycine + 0.03M K_2HPO_4, pH 9.2), and inoculating to Samsun tobacco, but few plants became infected (3/20). The success rate in transmission was not improved by attempting to stabilize the viral genome with a phenol extraction procedure (7). Frozen leaf samples lost infectivity within 3 days, and dilution of sap over 1:50 abolished the infectivity. However, greater efficiency in transmission was obtained when infected Samsun tobacco or *Gomphrena globosa* L. were used as source plants. The following plants were susceptible to FHV.

Chenopodium amaranticolor - Chlorotic local lesions appeared in 7-10 days (Fig. 6); the lesions extended in diameter and sometimes spread to veins, where subsequent necrosis led to premature leaf abscission.

Datura ferox Nees and *D. aegyptica* Vesl. - Oak leaf pattern and veinal necrosis (Fig. 3) developed in 7-14 days.

Gomphrena globosa - Chlorotic to water soaked lesions appeared in 7-10 days. These spread to the veins, causing veinal necrosis and premature leaf wilting, followed by systemic symptoms of necrotic lesions, dark veins and reduction in leaf size (Fig. 7).

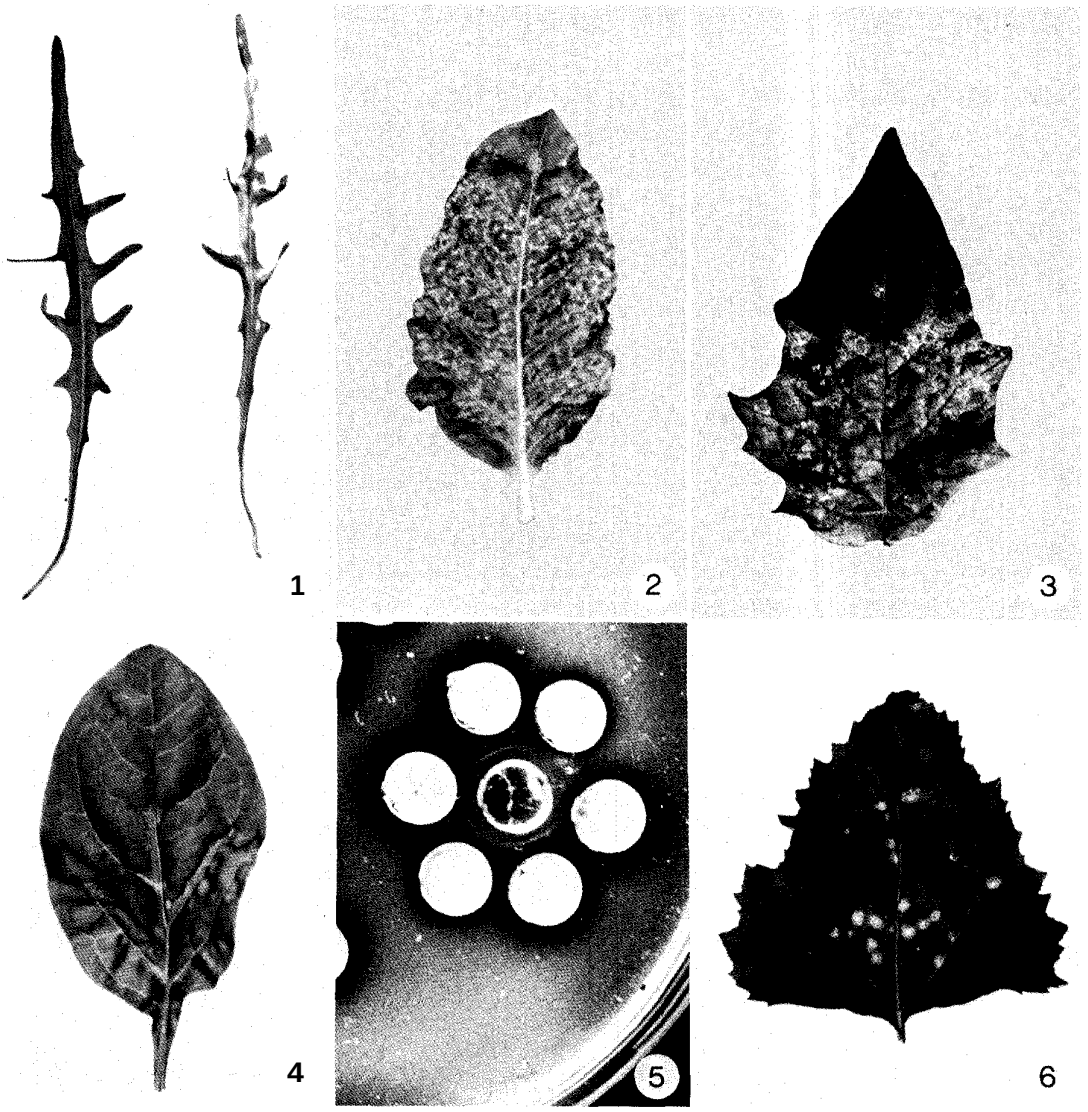

Fig. 1. Left, healthy fall hawkbit leaf; right, naturally infected leaf with chlorotic mosaic.

Fig. 2. Systemically infected Samsun tobacco leaf showing oak leaf pattern

Fig. 3. Systemically infected *Datura aegyptica* leaf showing necrosis and oak leaf pattern.

Fig. 4. Inoculated *Petunia hybrida* leaf showing veinal necrosis.

Fig. 5. Results of serological reactions in SDS-agar with HMV antisera in the center well: a) sap from FHV-infected Samsun tobacco, b) sap from healthy tobacco, c) sap from healthy fall hawkbit leaves.

Fig. 6. Local lesions on a *Chenopodium amaranticolor* leaf induced by FHV

Nicandra physaloides Gaertn. Necrotic spots were observed. Not all the inoculated or systemic leaves developed symptoms.

Nicotiana tabacum cvs. Samsun and White Burley - Necrotic lesions along the mid vein, ring spot lesions on the lower leaves or oakleaf patterns (Fig. 2) appeared in 7-10 days. A mild mosaic developed systemically.

Petunia hybrida Vilm. - Necrotic blotches (Fig. 4) along the main veins developed within 2 weeks.

Physalis pruinosa L. - Various degrees of leaf necrosis and leaf wilting were observed after 3-4 weeks.

No viral infectivity was recovered on back-testing of inoculated and uninoculated leaves of the following species: *Nicotiana glutinosa* L., *Phaseolus vulgaris* L. (7 cultivars),

Fig. 7. Systemically infected *Gomphrena globosa* plants showing stunted leaves with necrotic spots and veins.

Pisum sativum L. (5 cultivars), *Scopolia sinensis* Hemsl and *Solanum tuberosum* L. (8 cultivars).

The virus was not transmitted by three species of aphids, i.e., *Myzus persicae* (Sulzer), *Aulacorthum solani* (Kltb.), and *Macrosiphum euphorbiae* (Thomas), from infected Samsun tobacco to healthy tobacco in 3 different tests.

Electron microscopy. Negatively stained extracts from FHV-infected tobacco and fall hawkbit leaves contained very low concentration of short rigid rods, about 23 nm in diameter (Fig. 8). Measurement of the lengths of 328 particles revealed two main size classes of 140 nm and 160 nm (Fig.9).

Serology. Antisera to five isolates of TRV (8) and an antiserum to pea early browning virus, failed to react in SDS-agar with FHV-infected tobacco or 'fall hawkbit' sap. However, a specific reaction was observed (Fig. 5) to an antiserum prepared against HMV.

Discussion

Although FHV showed some resemblance to TRV, particularly in symptomatology, it did not react with several antisera prepared against European strains of TRV. Thus the virus in question is not TRV as had earlier been speculated (6). However, FHV reacted specifically with an antiserum to HMV, a newly described virus from another species of Compositae. In spite of the similarities to HMV, some differences in host range were observed with the 'fall hawkbit' isolate; e.g., it infected *Gornphrena, Datura, Physalis* and *Petunia* species which were not infected by HMV (1).

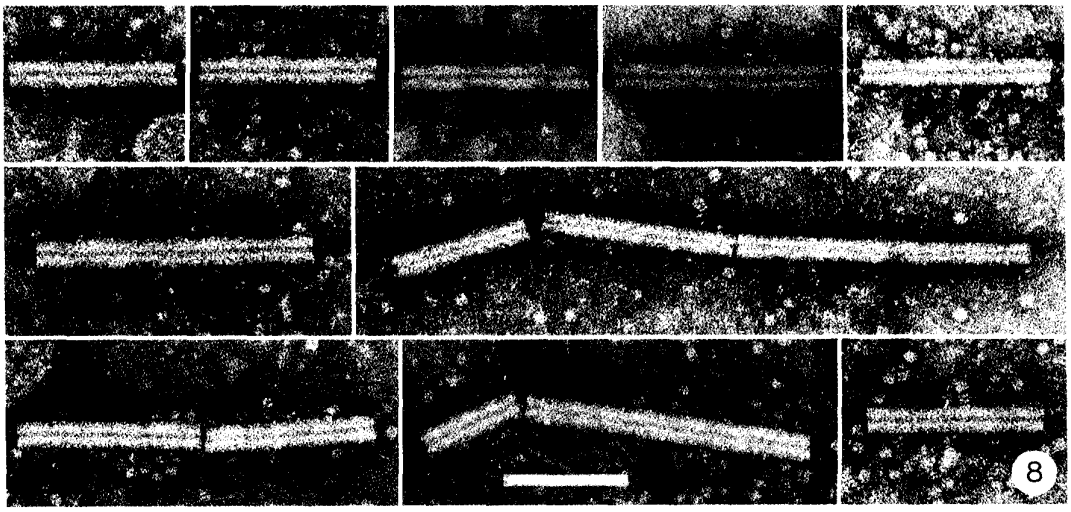

Fig. 8. Particles in leaf extracts of FHV-infected fall hawkbit negatively stained with 2% ammonium rnolybdate, pH 7. Bar = 100 nm.

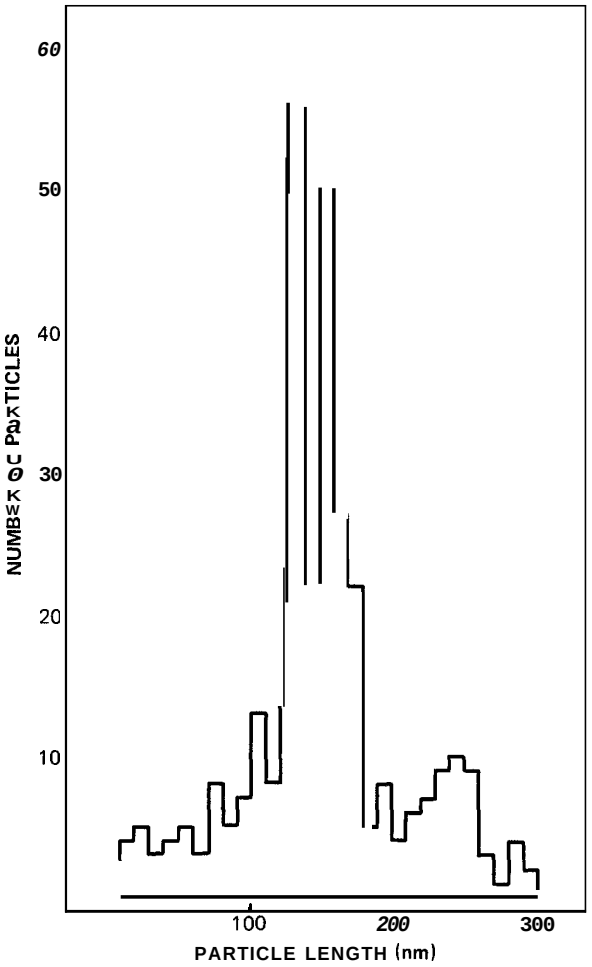

Fig. 9. Length distribution of particles found in negatively stained extracts of FHV-infected fall hawkbit.

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