EFFECT OF HYDROCARBONS AND CRUDE OIL CONTAMINATION ON THE SENSITIVITY OF FRENCH BEAN TO ALFALFA MOSAIC VIRUS

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Summary. – Determination of local necrotic lesions on primary leaves infected by alfalfa mosaic virus (AMV) revealed that hydrocarbons (HC) contamination of the substrate used for cultivation of French bean (*Phaseolus vulgaris* L., cv. Black Turtle Soup) caused a reduction of bean leaf area and an increase of plant sensitivity to AMV infection. On the other hand, superficial contamination of the leaves by crude oil caused an inhibition of lesion formation. Changes of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of extractable bean leaf proteins related to the cultivation substrate contamination by HC were also detected.

Key words: hydrocarbon contamination; crude oil; alfalfa mosaic virus; local necrotic lesions; French bean; polyacrylamide gel electrophoresis

Different stimuli, such as viruses, and chemical or mechanical injuries may evoke similar biochemical and ultrastructural changes in plants, e.g.hypersensitive reaction (Appiano *et al.*, 1981; Redolfi *et al.*, 1982; Conti *et al.*, 1991). Changes in soluble protein composition of plants during their reaction to different viruses (AMV, tobacco necrosis virus, tomato bushy stunt virus, tobacco mosaic virus), chemicals ($CuSO_4$, HNO_3), viroids and fungi have been described (Szcepanski and Redolfi, 1985; Conti *et al.*, 1991). The number of local lesions on plant leaves induced by viral infection is influenced by various factors (Jurík and Musil, 1973; Kvíčala, 1974; Musil and Jurík, 1974; Peters and Lebbink, 1975; Abu-Jawdah and Kummert, 1983).

The aim of the present study was to elucidate whether HC contamination of the cultivation substrate can cause changes of the number of lesions induced by AMV on primary leaves of French bean and of extractable proteins, or cannot.

French bean (*Phaseolus vulgaris* L., cv. Black Turtle Soup) was sown and cultivated in plastic discs (17 cm in diameter) in a greenhouse at 18 – 23 °C. Perlite (Keramické závody Košice, Factory Lozorno, Slovakia) and soil served as the substrate for plant cultivation.

The contamination of the substrate with crude oil was carried out 3 days before the infection of plants with AMV. The HC content was 97,500 mg per kg of perlite. Soil samples of the same origin containing 24,000, 11,200, 8,000, 5,700 and 3,200 mg HC per kg, respectively, were supplied by Research Institute for Crude Oil and Hydrocarbon Gases, Slovnaft Inc., Bratislava, Slovakia. Superficial contamination of primary bean leaves by crude oil was exerted 24 hrs before the AMV inoculation. Forty µl of crude oil were applied on each leaf (10 leaves in one experimental group). Crude oil was spread on the whole leaf surface by a glass stick. Five % crude oil emulsion in water made with 0.01% Tween 20 was used in another experimental group.

AMV isolate T6 (Gallo, 1980) was purified from pea leaves (*Pisum sativum* L.) by polyethylene glycol (PEG 6000) precipitation combined with high-speed centrifugation (40,000 rpm, 2 hrs). The virus was resuspended in 0.1 mol/l phosphate buffer pH 7.0. For inoculation, a virus dilution causing 5 – 8 lesions on the leaves of untreated control plant group was used (Jurík and Musil, 1971). Forty µl of virus inoculum per leaf were mechanically inoculated using a rubber sponge. Local necrotic lesions were counted 4 days after virus inoculation. The differences were evaluated statistically by the analysis of variance and by Student's t-test, and related to the estimated leaf area.

Separation of proteins of bean leaf extracts was performed by SDS-PAGE according to Laemmli (1970). The gels were silver-stained (Marcinka *et al.*, 1992).

Abbreviations: AMV = alfalfa mosaic virus; HC = hydrocarbons; PAGE = polyacrylamide gel electrophoresis; PEG = polyethylene glycol; SDS = sodium dodecyl sulphate

Table 1. Sensitivity of French bean plants cultivated in HC-contaminated substrates to AMV infection

Substrate	HC (mg/kg)	Average leaf area (cm^2)	Average number of lesions per leaf	Average number of lesions per cm^2
Perlite	97,500	10	17.2 ± 3.2	1.7
	0	19	7.3 ± 1.3	0.4
Perlite	97,500	10	11.5 ± 4.8	1.2
	0	19	4.3 ± 0.7	0.2
Soil	24,000	10	3.8 ± 3.0	0.3
	8,000	17	5.1 ± 2.8	0.3
	0	20	3.8 ± 2.2	0.2

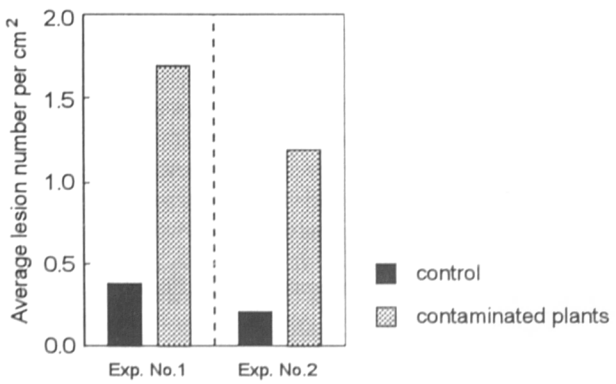

Fig. 1
Sensitivity of primary leaves of French bean cultivated in HC-contaminated perlite to AMV infection

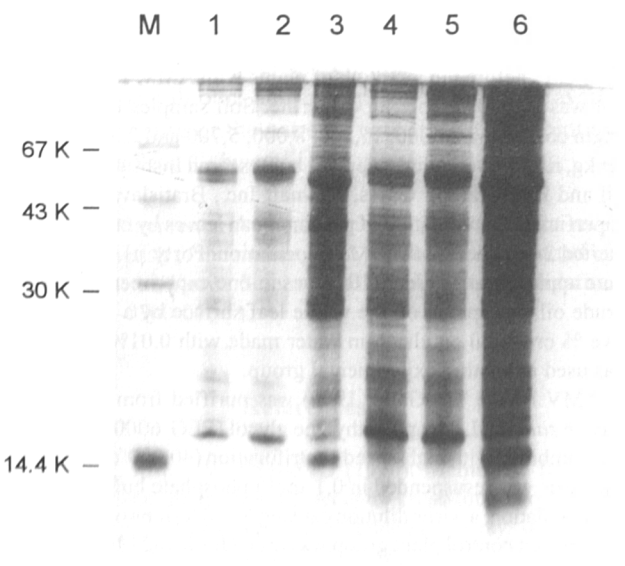

Fig. 2
SDS-PAGE of proteins extracted from primary leaves of French bean
The plants were cultivated for 14 days in perlite contaminated by 97,500 mg HC per kg (lanes 1,3,4,6). Control plants (lanes 2,5). Sample volumes of 0.5 µl (lanes 1,2,3) and 5 µl (lanes 4,5,6) were loaded onto gel. M_r markers: albumin (67 K), ovalbumin (43 K), carbonic anhydrase (30 K), α-lactalbumin (14.4 K) (lane M). The leaf area of plants was reduced by 30% (lanes 1,4) and 70% (lanes 3,6).

In the experiments, bean plants were cultivated in perlite contaminated by a high dose of HC (97,500 mg per kg) for 3 days before virus inoculation. Surprisingly, 4 – 6 times higher numbers of lesions were formed on primary leaves of these plants in comparison to control plants cultivated on non-contaminated perlite (Table 1, Fig. 1); the differences were statistically significant ($p = 0.05$). The leaf area of the test plants was reduced by 50% in comparison to control plants. When the test plants were cultivated for 14 days in soil contamined by a lower HC dose (24,000 mg per kg), no significant differences in mean number of lesions in comparison to control plants were found. However, the leaf area of test plants was reduced by 50% in comparison to control plants.

Extracts from primary leaves of French bean cultivated for 14 days in the contaminated soil containing 3,200, 5.700 and 11,200 mg HC per kg were run in SDS-PAGE. No protein pattern changes in comparison to control plants were detected. Also in plants cultivated for 50 days in soil containing 3,200 mg HC per kg no changes were detected. However, changes in protein pattern, mostly of quantitative nature, were found in plants sown and cultivated for 14 days in highly contaminated perlite (Fig. 2).

Changes in plant proteins resulting from plant cell damage by different chemicals, pathogenic factors or environmental stress have been observed (Henriquez and Sanger, 1982; Abu-Jawdah and Kummert, 1983; Redolfi, 1983; Repka, 1993). However, no reports on the effect of HC on lesion formation or on protein synthesis in virus-infected plants are known to date. We assume that binding of water by soil and perlite containing HC, and evaporation of HC gases affect the sensitivity of leaves to AMV infection, because the humidity itself significantly affects AMV multiplication in primary leaves of French bean (Jurík, 1972). To eliminate the effect of unknown soil factors, perlite was used as a model inert cultivation substrate. The contamination of cultivation substrate (perlite, soil) increased the sensitivity of primary leaves of French bean to AMV infection and caused the reduction of plant leaf area.

Already our initial studies showed a phytotoxic effect of the superficial contamination of leaves by crude oil substances and an inhibitory effect on the establishment of AMV infection (Fig. 3). The phytotoxicity of different crude oil derivatives will be an aim of another our study. An inhibitory effect of mineral oil sprays on the transmission of plant viruses has been reported in numerous field and laboratory studies, however, its mechanism is not yet clear. In low concentrations, the mineral oil probably acts just as a barrier preventing virus penetration into plant cells (Peters and Leblink, 1975; Gudin *et al.*, 1976; Vanderveken, 1977; Boiteau and Wood, 1982; Jurík, 1988).

The results of this work support the idea to use plants as model organisms for the estimation of degree of contami-

nation or decontamination of cultivation substrates (soil). Most attractive is the use of plants reacting sensitively and intensively to the presence of contaminants in the soil.

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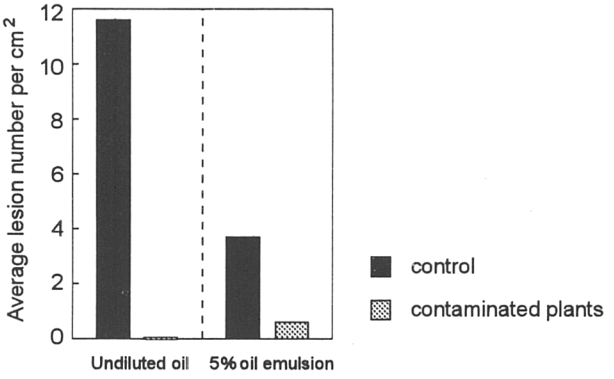

Fig. 3
Inhibition of lesion formation on primary leaves of French bean contamined by crude oil emulsion

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