Effects of Temperature on Infection of French Bean Leaves (Phaseolus vulgaris L.) by Lucerne Mosaic Virus

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Abstract. The effect of temperature on the number of lesions and the time of their appearance was studied by inoculating French bean leaves (Phaseolus vulgaris L. cv. Peršska) with lucerne mosaic virus either 24 or 48 h before or, 24 or 48 h after they were exposed to various temperatures. The temperatures tested were 23, 25, 27, 30, 33 and 36 °C.

Before and after such exposures the plants were kept in a constant temperature of 25 °C. By increasing the temperature before inoculation the number of lesions increased in comparison with the control. The optimal temperature for the maximum number of lesions is between 27 ° and 30 °C. There is no significant difference between those experiments when the exposure time was 24 h or 48 h before inoculation. The same temperatures applied for 24 or 48 h after inoculation have a decreasing effect upon the number of lesions formed by LMV on French bean leaves. The decrease is 30 to 75%. In this case the first necrotic local lesions appeared 42 h after inoculation when exposed to higher temperatures above 27 °C for 24 h, and 60 h after inoculation when exposed to these temperatures for 48 h. The shape of lesions varied a little in both cases as the pictures show.

The most important environmental factors that affect the infection and multiplication of viruses in plants are the temperature and light intensity (Kassanis 1952, 1957, Niemhaus 1957, Fridlung 1959, 1967, Sinya 1960, Francki 1967). Yarwood (1958, 1961) reported an increase in the number of local lesions in the inoculated leaves of French beans (Phaseolus vulgaris L. cv. Pinto) with tobacco mosaic virus when dipped in a water bath at 45—55 °C for a short time (in seconds) later than 3 h after inoculation. The majority of plants show increased susceptibility to infection when they are kept at a high temperatures before inoculation, but different viruses show various types of behaviour when plants are kept at high temperatures after inoculation (Kassanis 1957). Lucerne mosaic virus (LMV) is one of the viruses the concentration of which in various plants is different at various periods of time after inoculation (Ross 1941a, b). This paper describes some of the characteristics of a strain of lucerne mosaic virus isolated previously from red clover (Musil et al. 1966), with regard to the optimal temperature conditions for infection of French bean leaves on which this virus produces necrotic local lesions.

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Materials and Methods

Lucerne mosaic virus (LMV) used in these experiments was isolated from red clover plants (*Trifolium pratense* L.) and was maintained and propagated in broad bean plants (*Faba vulgaris* Moench.). This virus is very pathogenic for this plant causing local necrotic spots on leaves and systemic stem necrosis. The infected plants soon die. For this reason the virus was reinoculated for experimental purposes every two weeks for a new series of healthy broad bean plants.

For quantitative work and infectivity assays the primary leaves of the French bean (*Phaseolus vulgaris* L. cv. Perliska) were used. Inoculations were made by rubbing the upper part of leaves with a piece of plastic foam wetted with inoculum and held by a pincette. Undiluted and diluted sap with carborundum added to increase the number of lesions was used as the original inoculum. After inoculation the leaves were rinsed with tap water and the plants placed in experimental boxes for observation. Before and after the inoculations the test bean plants were kept in thermostatically controlled glass sided incubators for various lengths of time and in various temperatures. The necrotic lesions on leaves were counted after a lapse of 25, 28, 31 and 46 h in plants inoculated after being exposed to various high temperatures. In plants which were kept at various high temperatures after inoculation, the number of necrotic lesions were counted after 25, 28, 31, 42, 46 and 60 h. All plants in the experiments were kept at a constant temperature of 25°C. To compare the results of various experiments all tested plants in all repeated experiments were of the same age, 13—15 days after sowing in the summer and 16 to 21 days in the winter. In pilot experiments these plants proved to be most suitable for further experimentation according to the number of local lesions.

Results

The effect of various temperatures on the number of lesions was studied by inoculating French bean leaves either 24 and 48 h before or, 24 and 48 h after they were exposed to 23, 25, 27, 30, 33 and 36°C. Before and after such exposures the French bean plants were kept in a constant temperature of 25°C. The experiments were repeated 5 times and in each experiment 12 inoculated leaves were taken. The results are summarised in Figures 1—3 and show that for more lesion production the temperature before inoculation is most important. With the increase of temperature the number of lesions increases in comparison with the untreated control. There are some differences between experiments exposed for 24 or 48 h, but these are not significant. The highest number of lesions was obtained at 36°C (1150 lesions after a 24 h exposure and 1293 lesions after a 48 h exposure to this temperature), in comparison with 731 lesions in the untreated control. The differences are
Fig. 4. Leaves of French bean plants (*Phaseolus vulgaris* L. cv. Perlička) showing local necrotic lesions of lucerne mosaic virus (LMV): a) plants 24 h before inoculation kept at 23°C, b) plants 24 h after inoculation kept at 23°C, c) plants 24 h before inoculation kept at 30°C, d) plants 24 h after inoculation kept at 30°C.
Fig. 5. Leaves of French bean plants (*Phaseolus vulgaris* L. cv. Perlička) showing local necrotic lesions of lucerne mosaic virus (LMV): a) plants 24 h before inoculation kept at 33°C, b) plants 24 h after inoculation kept at 33°C, c) plants 24 h before inoculation kept at 36°C, d) plants 24 h after inoculation kept at 36°C.
highly significant in all temperatures used in comparison with the control experiments. The concentration of LMV in the sap of broad bean plants was low and in some of our dilution experiments the sap diluted at 1 : 100 was no longer infectious.

The effect of high temperature after inoculation was also studied in some detail. Leaves of plants kept at 23° C for 24 h after inoculation formed about half as many lesions as the control leaves (296 and 431 resp.). Leaves of plants kept at 30° C for 24 h after inoculation formed 321 lesions, the control leaves 592, and again, those kept at 33° C for 24 h after inoculation formed 383 lesions and the leaves of the control plants 741, in an average of 5 experiments. In the temperature of 36° C the differences were more pronounced, e.g. 161 lesions on treated leaves and 731 lesions on untreated ones. Exposure for 48 h, after inoculation, to the same temperature has a more pronounced effect and the number of lesions is much lower. The details are given in Fig. 1 — 2, where lesion counts after various lengths of time are given. The infectivity of LMV in crude sap and in various dilutions of the inoculum taken from broad bean plants was tested with each experiment. The results given in the Fig. 3 show the final number of lesions on inoculated leaves in comparison with the control plants of the same experiments. The number of lesions in control plants in each experiment is a 100 and the final number of lesions in the experiments is expressed in percentage of this number.

The first necrotic local lesions in the plants exposed before inoculation to various temperatures usually appeared after 24 h as in the control plants. When the plants were exposed to high temperatures after inoculation, the lesions appeared later, usually after 42 h, when the time of exposure was 24 h.

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**Fig. 1.** The growth in numbers of local lesions on the inoculated leaves of French beans (*Phaseolus vulgaris* L.) with LMV in various time intervals. The plants were exposed 48 h before (b.i.) and/or 48 h after inoculation (a.i.) to the shaked temperatures.
**Discussion**

Our results are similar to Kassaris's (1952) who found with all 5 viruses with which he experimented that pre-inoculation exposure at 36° C increased the susceptibility of plants to these viruses. Also Sinha (1960) working with red clover mottle virus (RCMV) found that the pre-inoculation treatment
of French bean plants at 36° C for 24 h increased the susceptibility of plants to infection, but by keeping them for a further 24 h returned them to the level of the untreated plants. In our experiments the relative number of lesions in comparison to the control was the highest in the pre-inoculation exposure to the temperatures of 27° C for 48 h, and 30° C for 24 h resp. With LMV and French bean plants the number of lesions in plants treated 24 or 48 h at this temperature were not significantly different. We can say that on the contrary the number of lesions after 48 h of pre-inoculation treatment was a little higher in all tested temperatures. KASSANIS kept his plants at a high temperature for a maximum of 4 days. In our experiments the plants were kept in the prescribed temperatures only for 24 or 48 h. Before and after such exposures the plant were in a constant temperature of 25° C. This, and the slight differences in the ages of plants used, may alter their physiological condition and their susceptibility. The post-inoculation treatment at high temperatures has an adverse effect on the total number of local lesions. The number of lesions is smaller and they appear later. The infection in this case spreads rather more into veinlets of locally infected leaves. It is not so strikingly localized as in the experiments when high temperatures were applied before the inoculation.

References

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Изучалось влияние температуры на количество и скорость образования местных поражений вызванных инокуляцией листьев фасоли (*Phaseolus vulgaris* cv. Перличка) вирусом мозаики люцерны. Листья инокулировали 24 или 48 часов перед или после применения различных температур. Испытывали температуры 23, 25, 27, 30, 33 и 36 °C. В остальное время растения находились в помещении с температурой 25 °C.

По сравнению с контролем количество местных поражений возрастало с повышением температуры. Максимум местных поражений наблюдался при 27—30 °C. В опытах с применением температур 24 и 48 часов перед инокуляцией различия между этими сроками не были достоверными. В случае применения тех же температур 24 и 48 часов после инокуляции количество местных поражений понижалось на 30—75%, при применении температур над 27° в течение 24 часов первые местные некротические поражения появились 42 часа после инокуляции, при их применении в течение 48 часов поражения появились лишь 60 часов после инокуляции. В обоих случаях форма поражений несколько варьировала.