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Effect of Temperature on Susceptibility of the Primary Leaves of *Phaseolus vulgaris* L. to Red Clover Mottle Virus

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Abstract. Red clover mottle virus isolated in Czechoslovakia was studied in relation to its reaction to varying temperature on primary French bean leaves (*Phaseolus vulgaris* L.) on which it forms local necrotic lesions. The plants were kept 24 or 48 h before, or 24 or 48 h after inoculation at the temperatures 23, 25, 27, 30, 33 and 36° C. After such exposures the French beans were kept at a constant temperature of 25° C. The lesions were counted at various intervals. In the experiment the optimal temperature for the maximum number of lesions seems to be 36° C 48 h before inoculation. The temperature above 25° C applied 24 h after inoculation seems to have a decreasing effect upon the number of lesions formed by RCMV on primary leaves of French beans and the lesions appeared several hours later, especially at 30, 33 and 36° C. The temperatures 27, 30 and 33° C applied 48 h after inoculation have a further decreasing effect on the number of lesions. The temperature of 36° C applied 48 h after inoculation has an inactivating effect upon RCMV inoculated on French bean leaves and no lesions appeared 5 days after inoculation.

The effect of temperature on the susceptibility of French bean leaves to infection with red clover mottle virus (RCMV) was studied by Sinha (1960b) in England, where this virus was discovered in red clover plants for the first time (Sinha 1960a). Since that time a distinctive strain of this virus was isolated from red clover in Holland (Bos and Maat 1965). Musil (1966) in Czechoslovakia isolated the same virus from red clover. Later Musil and Matisová (1967) reported this virus from various localities in Czechoslovakia. Musil and Lešková (1969a, b) studied the course of infection of this virus in some host plants and the effects of temperature upon this virus in vivo and in vitro. Because this isolate has many properties in common with the Dutch isolate of RCMV, the aim of our investigation was to study in more detail the reaction of the Czechoslovak strain of this virus to temperature on the French bean cv. Perlička of Czechoslovak origin, with special regard to the lesion formation and their number. The temperature seems to be one of the most important factors that affect the initiation and development of viruses in plants as stated by many authors (Kassanis 1952, 1957, Fridlung 1959, Nienhaus 1957, Yarwood 1958a, b, Bodnár and Kvíčala 1968).

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Material and Methods

Red clover mottle virus, an isolate from Western Slovakia was kindly given by Dr. Musil of our Institute and is marked in the collection as TpM25. In our experiments the original isolate was propagated in broad bean plants (*Faba vulgaris* MOENCH.) at weekly intervals. For infection assays and quantitative work the primary leaves of French bean (*Phaseolus vulgaris* L. cv. Perlička) were used. The methods used for inoculation and handling of plants were the same as described by BODNÁR and KVÍČALA (1968), only the counting of lesions was made at different intervals due to generally late appearance of the first lesions of RCMV on the inoculated primary leaves of French beans. Constant temperatures were provided in glass incubators. The lesions were counted 49, 52, 55, 69, 71, 91, 94 h and in certain cases after 5 days. In each experiment 6 plants (12 primary leaves) were inoculated and the experiment was repeated 5 times at weekly intervals. Altogether 30 plants (60 primary leaves) were used in each experiment.

For each experiment the same number of control plants were taken from the greenhouse with daily temperature variations from 17 to 30° C and after inoculation they were kept at 25° C. The number of lesions in the control was lower than in the experiments. In the majority of experiments the final counting of lesions was 69 h after inoculation; only in some cases when the lesions did not appear by this time the observation time was prolonged up to 5 days. Lesions after 4 or 5 days usually coalesced, further counting was therefore not possible.

Results

1. In the preliminary experiment primary leaves of French beans 9, 11, 13, 15, 17 and 19 days after germination were inoculated with red clover mottle virus and kept at the constant temperature of 25° C. After 48 h all inoculated leaves showed distinct necrotic local lesions. The number of lesions increased with the length of time after inoculation. 69 h after inoculation the total number of lesions on 16 leaves of 8 inoculated plants for each age group was 133, 266, 567, 892, 720 and 423. The average number for each leaf was 8·3, 16·6, 35·4, 55·7, 45·0, and 26·4. Leaves of 15 days old plants showed the greatest amount of lesions, therefore, in further experiments only this age group of plants was used. Tests were made from June to September. These preliminary experiments confirm the results of SINHA (1960b) and clearly show that the susceptibility of bean plants to infection by RCMV is influenced by physiological stage and age of plants.

2. The influence of varying temperature before and after inoculation on the infection of French bean leaves by RCMV.

The plants were kept at 23, 25, 27, 30, 33 and 36° C 24 or 48 h before or after inoculation. After this period they were kept for observation of symptoms in the greenhouse box at 25° C. The first local lesions appeared on the leaves of control and experimental plants 48 h after inoculation. Experiment shows that the exposure to the varying temperature ranging from 23 to 36° C 24 h before inoculation increased the number of local lesion on leaves, *i.e.* increased the susceptibility of plants to infection.

The statistical evaluation of results using t-test shows no significant differences between the final number of lesions at the temperatures of 23 and 25° C, and 23 and 27° C, highly significant differences ($P = 0{\cdot}01$) at the temperatures of 23 and 30° C, 23 and 36° C, and significant differences ($P = 0{\cdot}05$) at the temperatures of 23 and 33° C. In comparison to the control plants the differences between the final numbers of lesions are not significant at 23 and 33° C, highly significant at 25 and 30° C, and only significant at 25 and 36° C.

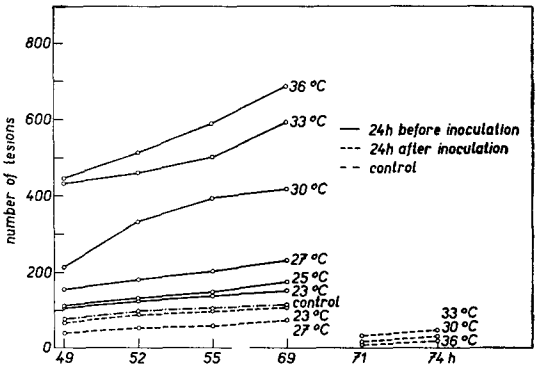

Fig. 1. The increase in number of lesions on the inoculated primary leaves of French beans (*Phaseolus vulgaris* L.) with RCMV at various time intervals and temperatures. Ordinate gives the mean number of lesions on 12 leaves, abscissa the time intervals of observation. Plants were exposed to the given temperatures 24 h before or 24 h after inoculation.

Increasing the length of pre-inoculation treatment to 48 h further increased the number of lesion (Fig. 2). There are no significant differences in the number of lesions are at the temperatures of 23 and 25° C, 23 and 27° C, 25 and 27° C, 30 and 33° C, 33 and 36° C, but highly significant differences ($P = 0{\cdot}01$) at the temperatures of 23 and 30° C, 23 and 33° C, 23 and 36° C, 25 and 36° C, 27 and 30° C, 27 and 36° C, 30 and 36° C. It shows only significant ($P = 0{\cdot}05$) differences at the temperatures of 25 and 30° C, 25 and 33° C, 27 and 33° C. Comparing the final number of lesions on plants treated 48 h before inoculation with the final numbers of lesions in the control plants the differences are not significant at the temperature of 23° C, but highly significant ($P = 0{\cdot}01$) at temperatures of 25, 27, 30, 33 and 36° C.

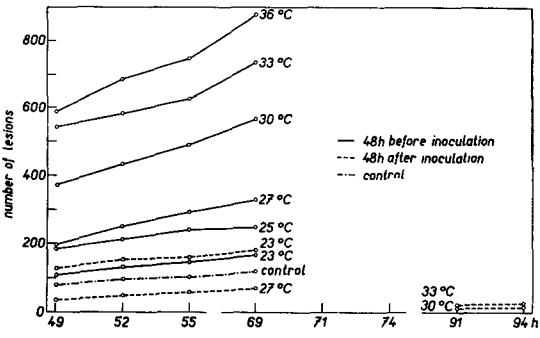

Fig. 2. The increase in number of lesions on the inoculated primary leaves of French beans (*Phaseolus vulgaris* L.) with RCMV at various time intervals and temperatures. Ordinate gives the mean number of lesions on 12 leaves, abscissa the time intervals of observation. Plants were exposed to the given temperatures 48 h before or 48 h after inoculation.

When the inoculated plants were kept at the temperature varying from 23 to 36° C 24 h after inoculation, the number of lesions decreased rapidly when the temperature was increased above 25° C. Only few lesions developed on leaves kept for 24 h after inoculation at 36° C. The experiment clearly shows that 24 h treatment after inoculation at the temperatures 30, 33 and 36° C rapidly decreased the number of lesions and delayed their appearance. The first countable

lesions in the experiment with these temperatures appeared 71 h after inoculation (Table 1*, Fig. 1). 48 h treatment of the inoculated plants at 30 and 33° C further decreased the number of lesions and their first appearance came after 91 h. No lesions appeared on plants kept 48 h after inoculation at the temperature of 36° C (Table 1). In the experiments using 24 h post-inoculation treatment in various temperatures, there were no significant dif-

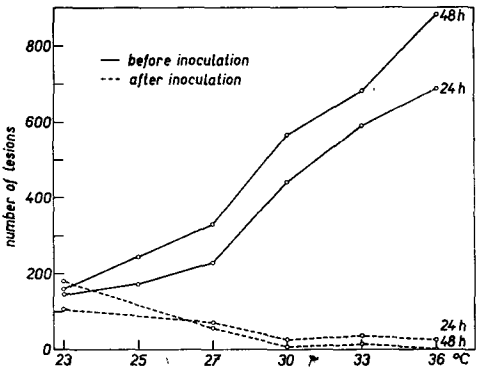

Fig. 3. The influence of various temperatures (abscissa) before and after inoculation on the number of local lesions (ordinate) on the inoculated primary leaves of French beans (*Phaseolus vulgaris* L.) with RCMV. The number of lesions is the final average out of 5 experments after 69, 74 or 94 h.

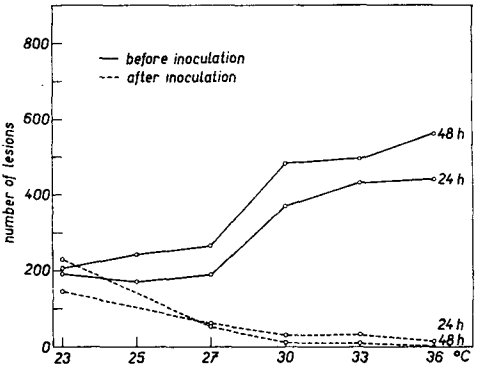

Fig. 4. The influence of varying temperature (abscissa) before and after inoculation on the number of local lesions (ordinate) on the inoculated primary leaves of French beans (*Phaseolus vulgaris* L.) with RCMV in relation to the number of lesions in control plants. The final number lesions after 69 or 74 or 94 h is given.

ferences between the numbers of lesions in plants kept at 23 and 27° C, 23 and 30° C, 23 and 33° C, 23 and 36° C, 27 and 36° C, 30 and 33° C, 30 and 36° C, 33 and 36° C, but highly significant differences at 27 and 33° C. Comparing the final number of lesions in this experiment with final number of lesions in the control plants the differences are not significant at 23 and 27° C, but highly significant at 30, 33 and 36° C.

48 h post-inoculation treatment in the given temperatures showed no statistically significant reductions in numbers of lesions at the temperatures of 23 and 27° C, 30 and 33° C, and highly significant differences in numbers of lesions at 23 and 30° C, 23 and 33° C, 27 and 30° C, 27 and 33° C. Comparing results of this experiment with the control plants there were no significant differences between the numbers of lesions at 23 and 27° C, but highly significant differences at 30 and 33° C.

The absolute number of lesions gradually increased with the length of time after inoculation (Fig. 3). The number of lesions gradually increased with increasing temperatures in which the plants were kept 24 or 48 h before inoculation. On the contrary post-inoculation treatment at the same temperatures gradually decreased the rather low number of lesions. In the experiment of 48 h post-inoculation treatment at 36° C no lesions appeared, even after 5 days. The number of lesions in all experiments in relation to the number of lesions on the control plants is given in Table 1 and Fig. 4.

* Table 1 see at the end of the issue.

Discussion

The pre-inoculation treatment at the temperature ranging between 23 and 36° C for 24 or 48 h increased the susceptibility of French bean leaves to RCMV and the highest number of lesions was obtained when the plants were exposed to 36° C for 48 h. In the experiments of SINHA (1960b) the pre-inoculation treatment at 36° C for 24 h increased the susceptibility of plants to infection, but keeping them for 48 h at this temperature returned it to the level of unheated plants. This is the only discrepancy with our experiments where the highest susceptibility measured by the number of lesions was obtained in keeping plants 48 h at 36° C, before inoculation with RCMV. Our results concerning the post-inoculation treatment at 36° C for 24 or 48 h are in accordance with SINHA's (1960b) experiments. No lesions were obtained when the plants after inoculation with RCMV were kept at 36° C for 48 h and then at 25° C for further 5 days. The lesions took longer to appear when the plants were kept at 30, 33 or 36° C 24 h after inoculation with RCMV. The same holds for 30 and 33° C and 48 h post-inoculation treatment. The much lower number of lesions in control plants in comparison with the plants kept 24 or 48 h before inoculation at the temperature of 25° C may be explained by the fact that the control plants before inoculation were kept in the greenhouse with varying temperature ranging from 17 to 30° C. The susceptibility of French beans to infection with RCM increased less rapidly when plants were kept 24 or 48 h before inoculation in the temperature between 23 and 27° C (no significant differences) and more rapidly between 27 and 36° C (highly significant differences), as seen in Figs. 3 and 4. The susceptibility due to the post-inoculation treatment at various temperatures decreased more rapidly between 23 and 30° C (statistically highly significant) and less rapidly between 30 and 36° C (not significant). MUSIL and LEŠKOVÁ (1969a) noted that the number of lesions on the inoculated leaves of beans by RCMV depended on the virus concentration, the age of plants, and on the environmental conditions, which agrees with our results. MUSIL and LEŠKOVÁ (1969b) moreover found with the same isolate of RCMV that it multiplies better and to high concentration in bean leaves at about 25° C and that at 20 and 30° C its concentration was lower. In our experiments temperatures above 27° C applied after inoculation lowered considerably the number of local lesions produced by this virus on bean leaves.

There are some differences in the reaction of various viruses to higher temperatures. BODNÁR and KVÍČALA (1968) working with AMV (lucerne mosaic virus), found the highest number of lesions on French bean leaves in the pre-inoculation exposure to the temperature of 27° C for 48 h or 30° C for 24 h. The number of AMV lesions in plants treated 24 or 48 h at 27 or 30° C are not significantly different. With RCMV the pre-inoculation treatment at high temperatures between 27 and 36° C for 48 hours produced more lesions than the treatment at the same temperature for 24 h; however, the differences are not significant. The appearance of the first local lesions on the inoculated primary leaves of French beans varied considerably according to the virus used. With AMV it is 24 h, with RCMV usually 48 h. The post-inoculation treatment at high temperatures for 24 h seems to have a similar effect on both these viruses causing local lesions in French bean

leaves. With AMV the total number of lesions is very low at 36° C and they took longer to appear. With RCMV similar results were obtained; however, the lesions appeared some hours later.

Acknowledgement

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B. A. Kvíčala, J. Bodnár, Virologický ústav SAV, Bratislava: **Vliv teploty na vnímavost primárních listů fazolu (*Phaseolus vulgaris* L.) k viru strakatosti jetele lučního.** — Biol. Plant. **13** : 273—278, 1971.

Autoři zjišťovali vliv teploty na počet a rychlost objevení se lokálních lezí na primárních listech fazolu, očkovaných izolátem viru strakatosti jetele lučního (RCMV) nalezeném v Československu. Fazole ve stáří cca 15 dní po vzejití byly 24 nebo 48 h před nebo po očkování pěstovány v teplotách 23, 25, 27, 30, 33 a 36 °C, později při konstantní teplotě 25 °C ve skleněných inkubátorech. Optimální teplota pro maximální počet lokálních lezí se zdá být 36 °C, pokud působila na rostliny 48 h před nákazou. Teploty nad 25 °Ç, při nichž jsou rostliny pěstovány 24 h po očkování, způsobují značné snížení počtu nekrotických lokálních lezí viru strakatosti jetele lučního na primárních očkovaných listech fazolu a léze se objevují později, zvláště při teplotě 30, 33 a 36 °C. Teploty 27, 30 a 33 °C, při nichž jsou rostliny po očkování pěstovány 48 h, působí další snížení počtu lokálních lezí tohoto viru na listech fazolu a teplota 36 °C, jíž byly rostliny vystaveny 48 h po očkování, způsobila, že na očkovaných listech fazolu se vůbec ne objevily leze ani po 5 dnech.