THE POSSIBLE USE OF ALTERNATING TEMPERATURES FOR THE REDUCTION OF VIRUSES IN PLANT TISSUE*

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The many successes of heat as a cure for plant virus diseases (Nyland and Goheen 1969) have obscured a lack of information on the principles of heat therapy (Kassanis 1957). The use of low temperatures as a therapeutic measure (Selsky and Black 1962) has had little attention, and the apparent failure of alternating temperatures to reduce virus infections has limited another approach to heat therapy. Recent experiments with tobacco mosaic virus (TMV) and other viruses have shown the possible usefulness of alternating extremes of diurnal temperature, and have re-emphasized the need for more theoretical information on the reactions of virus-infected plant tissues to various temperature regimes.

The emergence of heat-tolerant virus strains is generally recognized by practitioners of heat therapy (Nyland and Goheen 1969) and by workers with TMV (Holmes 1934; Lebeurier and Hirth 1966), but the extent of the changes likely to occur in a virus population after heat therapy has begun are seldom taken fully into account. These changes were indicated by experiments (Bald, unpublished data) in the phytotron at Canberra in 1963–64, which tested the effects of temperature on tobacco and *Nicotiana glauca* infected with TMV. Temperatures were either constant, or fluctuated 5°C between day and night. At 33 and 36°C the balance of strains in replicating virus populations was radically altered. Also the rate of virus multiplication increased with increasing temperature up to 36°C, which is often applied as a therapeutic treatment to many virus–host combinations. At the other extreme, low temperatures delayed virus translocation and systemic infection. Altogether, the Canberra experiments illustrated why heat therapy has not been successful with a number of stable viruses. They indicated that cold temperatures or an alternation of hot and cold should be further studied as a basis for heat therapy. The latter alternative was chosen.

Results and Discussion

The trials at alternating temperatures were made in two controlled-temperature cabinets at the University of Tasmania during 1970–71. Only one cabinet at a time was available. First, temperatures of 30 and 13°C were alternated between a 12-hr day and a 12-hr night. The virus was TMV, the variety of tobacco was Hickory Pryor, and plants were inoculated immediately before treatment. Virus multiplied rapidly and symptoms appeared in 4 days, which was at least 1 day less than would be expected at a mean temperature around 25°C under ordinary greenhouse conditions. In the actual checks held in a cool greenhouse at daily mean temperatures,

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20–23°C, the incubation periods were 8–11 days. The results at 36–13°C agreed with the suggestion that the high and low temperatures would compensate for each other (Kassanis 1957).

The opposite effect began to appear at temperatures alternating between 40 and 10°C. The infectivity of virus in inoculated leaves remained below that developing in the cool-greenhouse checks. Translocation and the appearance of systemic symptoms were delayed. In these trials and also those at more extreme alternating temperatures there was evidence of initial virus multiplication 1–4 days after temperature treatment began. Such increases were followed by rapid decreases in virus concentration.

In a number of trials newly infected plants were given approximately a 12-hr day at 44°C, and a 12-hr night at 6°C. After 3–14 days virus concentrations in inoculated leaves were measured by local lesion assays of purified, clarified, or crude virus suspensions from inoculated leaves. The results depended partly on the speed of transition between the diurnal extremes of temperature.

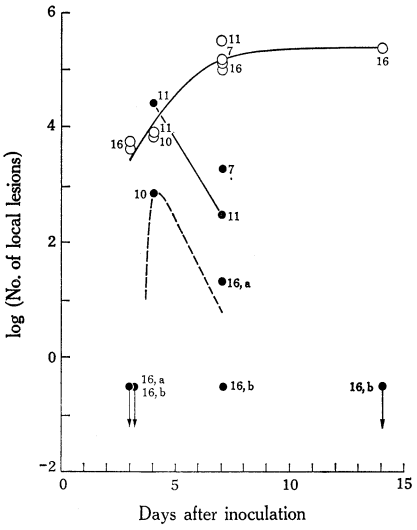

Fig. 1.—Infectivity of virus suspensions from inoculated leaves in four trials (Nos. 7, 10, 11, 16) with TMV in tobacco at 44–6°C. Values for infectivity were derived from numbers of lesions per half leaf of *Nicotiana glutinosa*, inoculated with variously diluted virus suspensions from sampled tobacco leaves. Lesion numbers were adjusted to equivalence with a standard inoculum, defined as the virus extracted from 1 g of tissue in 1 ml of water or buffer solution. The upper continuous curve is drawn through values (○) for cool-greenhouse checks. The scattered points (●) and arrows, all but one being below the curve, represent infectivity in temperature-treated tissues. Arrows represent zero lesion readings.

Infectivity data for inoculated leaves from four trials at 44–6°C are summarized in Figure 1. Only two treatments are considered: (1) an uninterrupted series of alternating temperatures; and (2) cool-greenhouse checks. Readings for the checks were adjusted to bring them on one curve, and values for temperature-treated plants were plotted accordingly. Two trials, 7 and 11, were in a cabinet with artificial light, in which the changeover between the extremes of temperature occupied altogether about 5 hr of the 24; and reduction of virus concentration in inoculated leaves at 7 days was about 10^{-2} or 10^{-3}. Trials 10 (4 days only) and 16 (14 days) were in a cabinet with natural daylight in which the change of temperature was much faster, and reductions of virus concentrations were 10^{-4}–10^{-6}.

In experiment 11 infectivity at 4 days was significantly higher in the heat-treated than in the check leaves but on the seventh day it was about one-thousandth that of the checks (Fig. 1). An early rise and subsequent fall of infectivity is suggested

for trials 10 and 16 by a dotted line. A similar rise and fall was apparent whenever positive infectivity readings were obtained from inoculated leaves of temperature-treated plants during the early period of treatment.

These extensive reductions of TMV concentration in treated tissues apply to plants inoculated immediately before treatment began. They do not necessarily apply to chronic infections. Chronic infections in several virus–host combinations were treated concurrently in these trials, with some apparent success; but to establish the usefulness of alternating extremes of temperature as a therapeutic measure large-scale trials will be needed. Also, more information should be collected on the balance between virus multiplication, degradation, and translocation.

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