Effect of Temperature on Symptoms of Tobacco Mosaic Virus and Movement of Photosynthate in Nicotiana glutinosa

Katie Helms and I. F. Wardlaw

Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra City, A.C.T. 2601, Australia.

We thank W. J. Müller of the Division of Mathematics and Statistics, CSIRO, for statistical treatment of the data and Ms. A. Andruska for efficient technical assistance.

Accepted for publication 27 September 1976.

ABSTRACT

HELMS, K., and I. F. WARDLAW. 1977. Effect of temperature on symptoms of tobacco mosaic virus and movement of photosynthate in Nicotiana glutinosa. Phytopathology 67: 344-350.

When single leaves of either entire or partially defoliated plants of *Nicotiana glutinosa*, were inoculated by rubbing or by jet-injection with tobacco mosaic virus (TMV), systemic symptoms developed at day/night temperatures of 33/28, 30/25, 27/22, 24/19, and 21/16 C. With both methods of inoculation, systemic symptoms developed earlier in plants kept at the higher temperatures than in those kept at the lower temperatures. When inoculation was by rubbing, systemic symptoms also developed in a greater percentage of plants kept at the higher temperatures than at the lower ones. Jet-injection and also partial defoliation promoted development of systemic symptoms at the lower temperatures. In partially defoliated plants, net photosynthesis decreased with increase in temperature from about 22 C to 40 C. Moreover, accumulation of dry matter per unit leaf area (storage) decreased with increase in temperature from 24 C to 36 C. As a consequence, translocation from the leaf (the difference between net photosynthesis and storage) did not change significantly with increase in temperature. Thus, there was no evidence to support the generally accepted view that systemic symptoms develop only at temperatures above 30 C and there was no evidence that a temperature of 30 C is critical in translocation.

It is accepted generally that the common strain of tobacco mosaic virus (TMV) induces systemic symptoms in mechanically inoculated plants of *Nicotiana glutinosa* kept at temperatures above 30 C (e.g., 1, 10, 13). We are aware of one report which states that occasional plants developed systemic symptoms when the highest temperature recorded on any day was 29 C (17). Since evidence from other hosts suggests that long-distance movement of TMV is correlated with movement of photosynthetic assimilates (2, 3), a temperature of about 30 C may be critical to those processes which contribute to the movement of carbohydrate; i.e., photosynthesis, the subsequent loading of carbohydrate into the phloem and the translocation of carbohydrate within the phloem.

Parallel work (4) has indicated that long-distance movement of TMV in *N. glutinosa* occurs in the phloem. In this paper, we have examined the effect of temperature both on the movement of TMV, as expressed by the development of systemic symptoms, and on the processes which contribute to the movement of photosynthate. We were particularly interested in the anticipated critical range of temperatures between 27 C and 36 C.

In an attempt to promote the long-distance movement of virus and the subsequent development of systemic symptoms, some test plants were partially defoliated and some were inoculated with a high level of inoculum (9) by jet-injection. The rationale for partial defoliation and jet-injection was as follows. Firstly, if the long distance movement of virus is correlated with movement of photosynthate, the long distance movement of virus and the subsequent development of systemic symptoms may be promoted in a simplified experimental system in which a single source of assimilate, as well as virus, supplies a single but distant sink. Partial defoliation ensured that there was only one source of virus as well as photosynthate and although there was some development of lateral buds during the course of the experiment, the roots constituted the only major sink for a short period after inoculation. Secondly, since entry of virus into the phloem is likely to be necessary for long distance movement and development of systemic symptoms (3, 4, 17) we attempted to promote this by jet-injection using a gun similar to that used previously for the inoculation of sugar beet with curly top virus (12). With this technique a fine stream of a suspension of virus can be passed under pressure through the midvein of a leaf. If some of the virus is inserted directly into the phloem, it might move long distances without prior multiplication; alternatively, it might first multiply in cells closely adjacent to the phloem before moving long distances within the phloem.

MATERIALS AND METHODS

Plant growth and inoculation with virus.—Plants of *Nicotiana glutinosa* L. were grown in 10-cm diameter pots in a mixture of perlite and vermiculite in the Canberra phytotron, under natural light with 8-hr

day/16-hr night temperatures of 27/22 C. Plants were watered with the standard phytotron nutrient solution [based on Hoagland's No. 2 solution (5)] in the morning and with water in the afternoon. Eight or 9 wk after the seeds were sown, just prior to the appearance of the influorescence, one nearly-expanded leaf near the top of the stem of an entire plant was inoculated, or the plant was decapitated and defoliated (partially defoliated plants), leaving one upper leaf for inoculation as in the entire plants. The leaves were inoculated by rubbing with

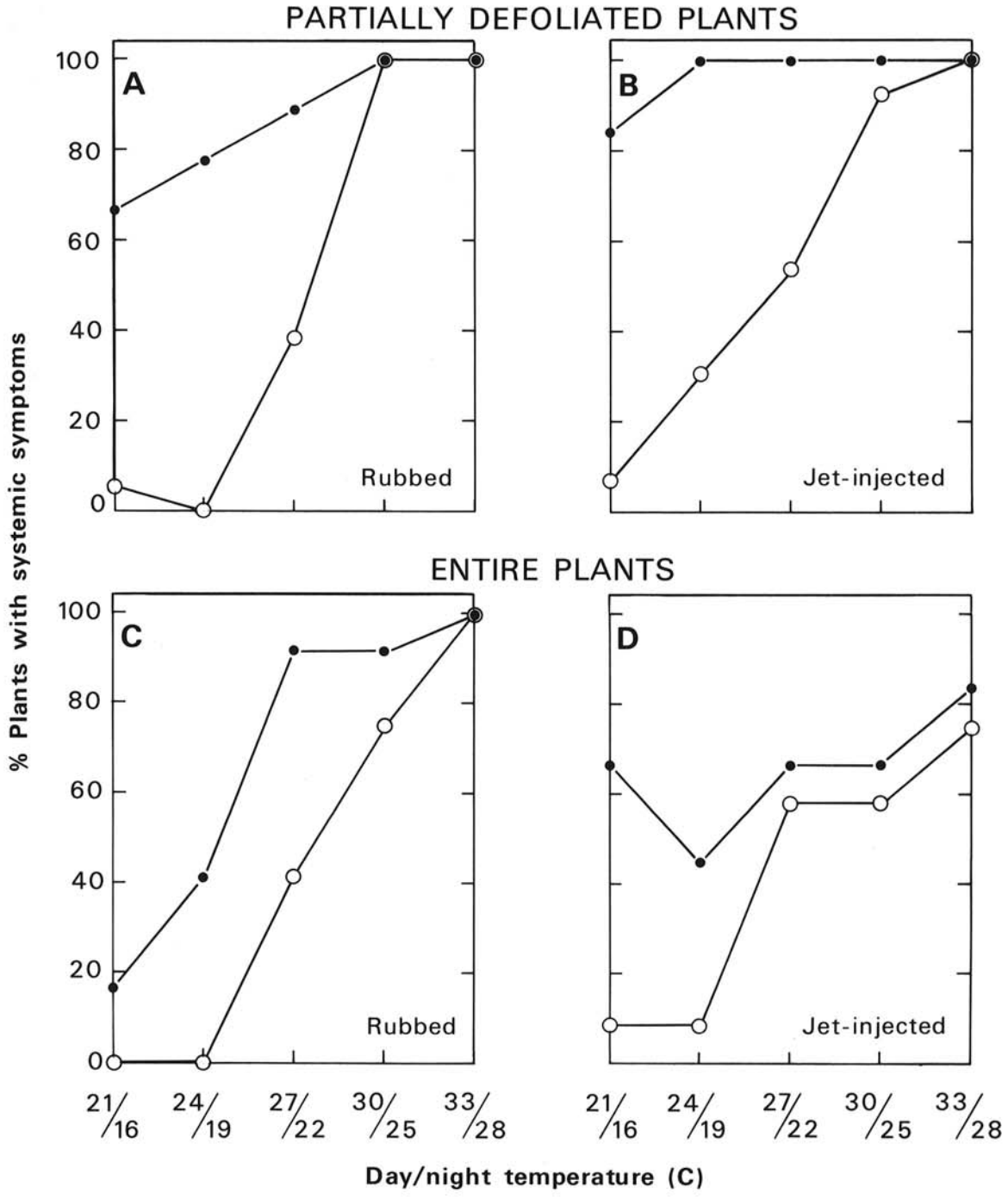

Fig. 1-(A to D). Effect of day/night temperature regimes on development of systemic symptoms of tobacco mosaic virus infections in plants of *Nicotiana glutinosa*. Partially defoliated plants and entire plants were inoculated on one upper leaf either **A)** and **C)** by rubbing leaves, or **B)** and **D)** by jet-injection. Legend: o——o represents data at 10 days after inoculation; and •——• data at 42 days after inoculation.

strain UI TMV (30-40 μg/ml) or twice jet-injected (5 mg/ml) in the abaxial, proximal end of the midvein of the lamina of the leaf using a Panjet gun (F. H. Wright Dental Mfg. Co. Ltd., Kingsway West, Dundee, Scotland), which normally is used in dental and veterinary work. After rubbing or jet-injection, plants were placed under natural light at various temperatures. At intervals of 2-3 days, during a period of about 42 days, plants were examined for the development of systemic symptoms.

Development of systemic symptoms in partially defoliated and entire plants in relation to temperature.—In Experiment I, development of systemic symptoms was examined in plants inoculated as described and subsequently kept at day/night temperatures of 33/28, 30/25, 27/22, 24/19, or 21/16 C. Partially defoliated plants were tested between August and November and entire plants during May and June. The tests were repeated several times and on each occasion two to five plants were kept after inoculation at each temperature condition. For inoculation by rubbing, the total numbers of plants examined at each temperature condition were 18 and 12 for partially defoliated and entire plants, respectively, whereas for jet-injected plants the numbers were 13 and 12 for partially defoliated and entire plants, respectively.

In Experiment II, development of systemic symptoms was examined in partially defoliated and entire plants which were derived from the same sowing and which were inoculated by rubbing. The experiment was done during July and August. After inoculation, plants were kept at 21/16 and 24/19 C. For both partially defoliated and entire plants, there were five plants at each temperature condition. The experiment was repeated once.

Data were summarized as contingency tables and significances were obtained using the chi-square test.

Net photosynthesis, dry weight changes, and export of ^{14}C-photosynthate.—Carbon dioxide exchange in the attached leaf of a partially defoliated plant was examined by enclosing the mid region of the leaf in a water-cooled Perspex (Plexiglas) assimilation chamber 2 × 5 cm in cross section. The differential in CO_2 concentration of an air stream, before and after passing over the leaf, was determined with a Grubb-Parsons infrared gas analyzer (Model SB2). Air flow rates ranging from 4 to 5 liters min^{-1} were such that the maximum difference in CO_2 concentration was no greater than 30 μliters/liter. All photosynthetic measurements were made in an artificially illuminated cabinet (11), with an irradiance on the leaf of 2,152 lux [= 64 Wm^{-2} (400 - 700 nm)]. Leaf temperature in the chamber was monitored by a thermocouple placed against the underside of the leaf.

Dry weights per unit area of leaf were determined on 8.4-mm diameter leaf disks, or whole leaves, which were oven dried at 80 C for 48 hr and then allowed to equilibrate under room atmospheric conditions prior to weighing.

Carbon 14 labeled CO_2 generated from 50 mg of $BA^{14}CO_3$ containing 628 μCi of ^{14}C was recirculated over

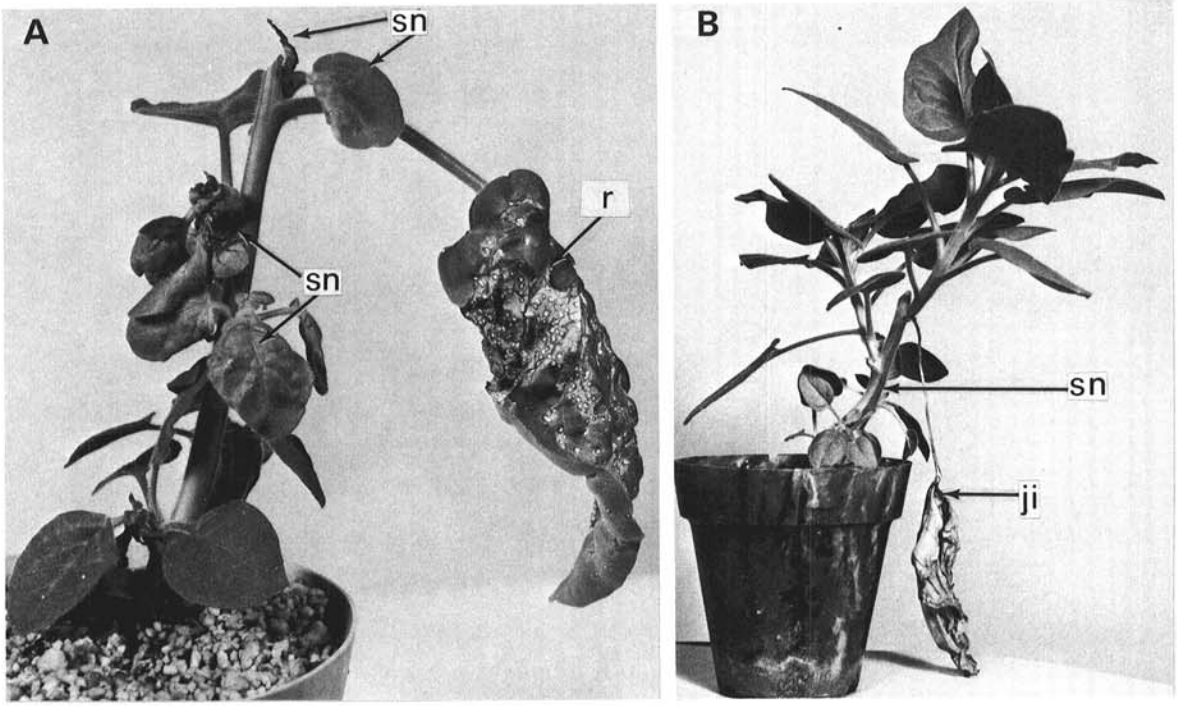

Fig. 2-(A, B). Systemic symptoms of tobacco mosaic virus in plants of *Nicotiana glutinosa* kept at temperatures below 30 C after inoculation. The plants were grown at day/night temperatures of 27/22 C and decapitated and defoliated except for one upper leaf, which was inoculated. **A)** The plant was inoculated by rubbing, kept at 21/16 C, and photographed 27 days later. **B)** The plant was inoculated by jet-injection, kept at 27/22 C, and photographed 19 days later. Legend: r = rubbed leaf; ji = jet-injected leaf; and sn = systemic necrosis of leaf or stem.

the upper leaf (of a partially defoliated plant) enclosed in the Plexiglas assimilation chamber. After a 2-min period, excess $^{14}CO_2$ was removed from the system by diverting the gas stream through soda-lime for 2 min before returning to a normal (nonlabeled) air flow. The movement of ^{14}C out of a leaf then was monitored continuously with a Geiger-Muller tube inserted through the base of the Plexiglas assimilation chamber just below the leaf, in a manner similar to that described by Hofstra and Nelson (8).

In Experiment III, data for net photosynthesis were obtained for the upper leaf of each of five plants, maintained in a cabinet with air temperature of 27 C, while leaf temperature was raised slowly from 15 to 43 C. At each temperature, data were recorded when photosynthesis had stabilized.

In Experiment IV, measurements were made of both the rate of dry weight accumulation per unit leaf area (storage) and net photosynthesis for a range of temperatures (24-36 C) during a period of 48 hr in continuous light. In this experiment, the whole test plant was exposed to the specified temperature. For each temperature, dry weight samples were taken from upper leaves at the beginning and end of the 48-hr period. Each determination was the mean of eight replicate leaves. Measurements of photosynthesis were made during the 48-hr period, on the same leaves as those used for the final dry weight determination. Some measurements were repeated on the same leaves at successive intervals so that small time-course changes in photosynthesis would be included in determinations of the mean photosynthetic rate for the period. Translocation per unit area of leaf was assessed from the difference between net photosynthesis and the rate of dry weight accumulation (storage).

In Experiment V, translocation was compared in plants that had been adapted to two different temperature conditions during a period of 4 days. Sixteen partially defoliated plants were kept for 4 days in each of two naturally lit glasshouses maintained at day/night temperatures of 27/22 C and 33/28 C, respectively. Measurements of dry weights were made on the leaves of eight plants in each glasshouse at the start of the 5th day,

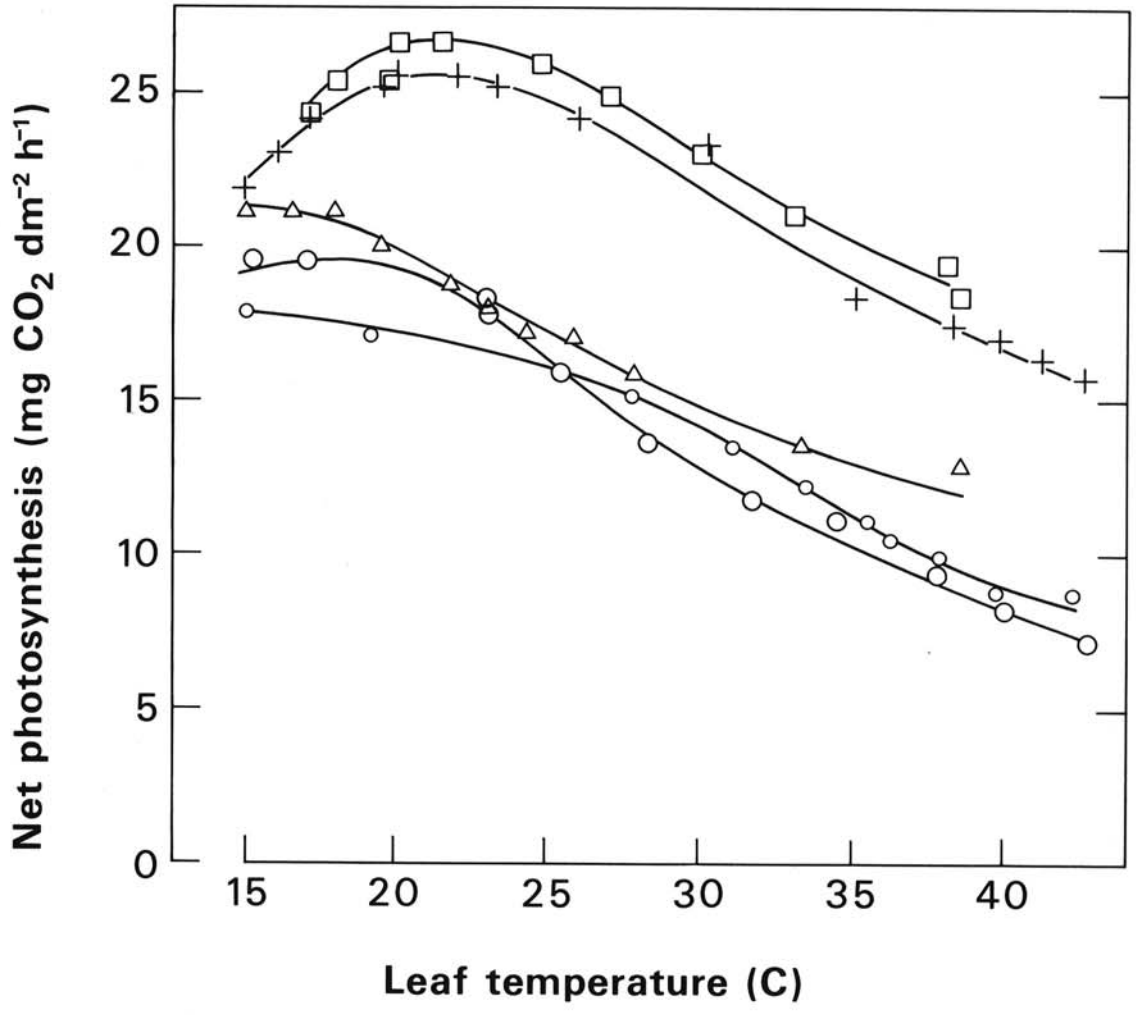

Fig. 3. Effects of temperature on photosynthesis in the upper leaf of partially defoliated plants of *Nicotiana glutinosa*. Data are for five individual plants.

and on the remaining eight plants 7.5 days later. Measurements of photosynthesis were made on the latter plants throughout the day. For these measurements the plants were transferred temporarily to artificially lit cabinets maintained at the same temperatures as the glasshouses and with standard light of 2,152 lux. This intensity approximated that in the glasshouses when somewhat cloudy conditions reduced light intensity. Translocation was determined as in the previous experiments.

In Experiment VI, measurements were made of the rate of export of ^{14}C from the upper leaf. Two sets of eight plants, one at 27 C and the other at 36 C, were kept for a period of 56 hr under continuous light. Export of ^{14}C and photosynthetic rates were measured at various times during this period and leaves were sampled for dry weight determinations 1 hour after the ^{14}C and photosynthesis measurements were made.

RESULTS

Temperature and development of systemic symptoms in partially defoliated and entire plants.—In Experiment I, partially defoliated and entire plants were inoculated between August and November and between May and June, respectively. Whether or not the plants were partially defoliated or entire, and whether or not they were inoculated by rubbing or by jet-injection, some plants at all temperature conditions developed systemic symptoms (Fig. 1). With plants inoculated by rubbing (Fig. 1-A, C), a greater percentage developed symptoms within 42 days at 33/28 and 30/25 C (higher temperatures), than at 24/19 or 21/16 C (lower temperatures) ($P = 0.001$). With jet-injected plants (Fig. 1-B, 1-D) the percentage which developed systemic symptoms within 42 days at the lower temperatures was almost as great as at the higher and the difference was not significant. With both methods of inoculation, a greater percentage of plants developed systemic symptoms within 10 days at the higher temperatures than at the lower ($P = 0.001$); i.e., systemic symptoms developed earlier at the higher temperatures than at the lower. At the lower temperatures, systemic symptoms developed earlier in jet-injected plants than in plants inoculated by rubbing (Fig. 1-B, 1-D c.f., Fig. 1-A, 1-C), ($P = 0.05$), they developed in a greater percentage of jet-injected plants than in plants inoculated by rubbing ($P = 0.05$), and they developed in a greater percentage of partially defoliated than entire plants (Fig. 1-A, 1-B c.f., Fig. 1-C, 1-D), ($P = 0.001$).

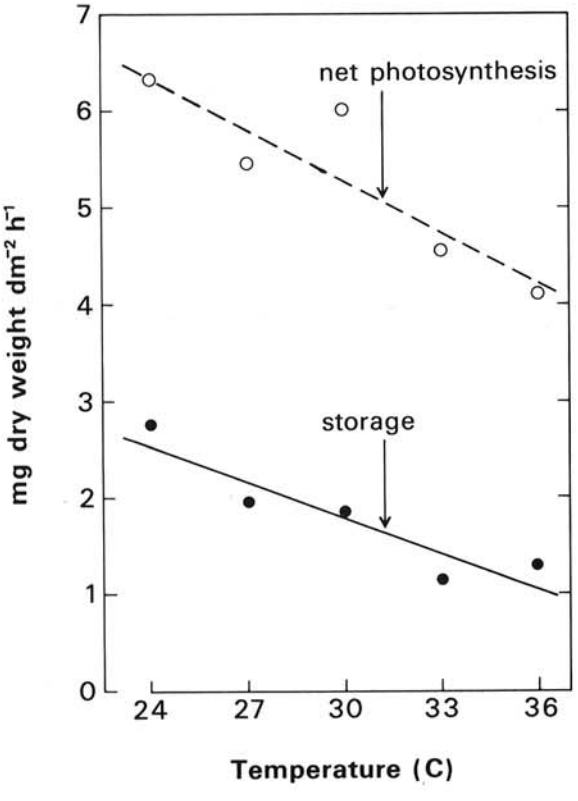

Fig. 4. Effects of temperature on net photosynthesis and dry weight accumulation (storage) in the upper leaf of partially defoliated plants of *Nicotiana glutinosa*. The fitted lines are y = 10.568–0.176x for photosynthesis and y = 5.496–0.123x for storage.

The purpose of Experiment II was to confirm with plants derived from the same sowing and inoculated (by rubbing) at the same time, that at the lower temperatures a greater percentage of plants developed systemic symptoms when partially defoliated rather than when entire. Systemic symptoms appeared between 14 and 23 days after inoculation. More partially defoliated plants (85%) than entire plants (45%) became systemically infected ($P = 0.01$). The results were consistent with those of Experiment I.

Symptoms.—In plants inoculated by rubbing and kept at 33/28 C, symptoms were essentially as described previously (13). Necrotic lesions developed only occasionally in the inoculated leaf; systemic symptoms developed in the stem and axillary shoots and the plants soon became completely necrotic. At 30/25 and 27/22 C, local lesions which formed in the inoculated leaf usually coalesced and systemic necrosis extended down the petiole and stem. At 21/16 C, local lesions developed in the inoculated leaf, whereas vein-clearing, chlorosis, and necrosis developed in the axillary shoots (Fig. 2-A); in partially defoliated plants, but not in entire plants, necrosis developed subsequently in the main stem.

In jet-injected plants kept at 33/28 C, systemic necrosis developed in the inoculated leaf close to the point of injection, and then in the main stem or axillary shoots. At 30/25 C and at lower temperatures, necrosis developed close to the point of injection and then extended both into the inoculated leaf and along the underside of the petiole and down the stem (Fig. 2-B). Sometimes, necrosis also developed in the stem above the injected leaf and in axillary shoots. Sometimes, the only evidence of systemic infection (other than in the inoculated leaf and petiole) was a few small lesions in the upper leaves. Occasional jet-injected plants did not become infected.

At the lower temperatures, for both inoculation by rubbing and by jet-injection, systemic symptoms tended to be more severe in partially defoliated plants than in entire plants.

Confirmation that the observed systemic symptoms were associated with infections of TMV was obtained by

inoculating test samples to leaves of *N. glutinosa,* which subsequently developed typical local lesions.

Net photosynthesis.—In Experiment III, net photosynthesis steadily decreased with increase in temperature from about 22 C to 40 C (Fig. 3). Consequently, there was a slight decrease in photosynthesis with rise in leaf temperature through the critical range from 27 C to 36 C.

Since it has been shown that defoliation alters the rate of photosynthesis in plants such as alfalfa (6), net photosynthesis was examined in a nearly-expanded leaf of *N. glutinosa* before and after the plant was defoliated. Results provided no evidence that defoliation altered net photosynthesis at the light level used in these experiments.

Translocation.—In Experiment IV, an examination was made of changes in net photosynthesis and in the rate of dry weight accumulation in leaves (storage) of plants exposed to temperatures ranging from 24 C to 36 C (Fig. 4). Net photosynthesis decreased significantly with increase in temperature ($r = 0.897$; $P = 0.01$) and the rate of storage also decreased significantly with increase in temperature ($r = 0.921$; $P = 0.01$). There was no significant difference between the slopes of the regression lines for photosynthesis and storage. This indicates that the rate of translocation, which was derived from the difference between net photosynthesis and the rate of storage, did not change significantly with temperature.

Two further tests yielded similar results. In the first, data for net photosynthesis and dry weights (d. wt.) were obtained for five plants kept under experimental conditions similar to the above but at temperatures of 27 C and 36 C only. In agreement with data in Experiment II, both net photosynthesis and storage were greater at 27 C than at 36 C, and translocation at 27 C (4.85 ± 1.04 mg d.wt. dm^{-2} hr^{-1}) was not significantly different from that at 36 C (5.04 ± 0.25 mg d.wt. dm^{-2} hr^{-1}). In the second, successive measurements of net photosynthesis and dry weight were made on two sets of eight plants kept at 27 C and 36 C, respectively, during a period of 56 hours; otherwise, experimental conditions were as for Experiment II. At neither temperature was there a significant trend in net photosynthesis with time. The mean rate of photosynthesis was 9.9 mg d.wt.dm^{-2} hr^{-1} at 27 C and 8.8 mg d.wt. dm^{-2} hr^{-1} at 36 C. From the slopes of regression lines fitted to plots of dry weight against time, the rate of storage was 3.94 mg d.wt. dm^{-2} hr^{-1} at 27 C, and 1.87 mg d.wt. dm^{-2} hr^{-1} at 36 C. Thus, the translocation rate at 27 C (5.96 mg d.wt. dm^{-2} hr^{-1}) was not significantly different from that at 36 C (6.94 mg d.wt. dm^{-2} hr^{-1}).

A similar response also was evident in Experiment V, in which plants received 4 days pretreatment, at either 27/22 C or 33/28 C, before measurements were made. Net photosynthesis was higher at 27 C than 33 C (11.5 and 9.5 mg d.wt. dm^{-2} hr^{-1}, respectively), and the rate of storage also was greater at 27 C than 33 C (7.3 and 5.7 mg d.wt. dm^{-2} hr^{-1}, respectively). Thus, the translocation rates were similar at 27 C and 33 C (4.2 and 3.8 mg d.wt. dm^{-2} hr^{-1}, respectively).

The experiments show that translocation did not change significantly with increase in temperature from 24 or 27 C to 33 or 36 C.

Export of ^{14}C-labeled photosynthate.—Although measurements of ^{14}C following pulse labeling are sometimes used to provide comparative information on rates of translocation of carbohydrate (8), the data can be difficult to interpret because of differences in the pool size through which the ^{14}C must pass prior to export in the vascular bundles. In Experiment VI where export of ^{14}C was measured after plants were preconditioned for between 6 and 48 hours at 27 C or 36 C before they were exposed to $^{14}CO_2$, the mean rate for ^{14}C export was more rapid at 36 C than at 27 C (7.8% and 5.1% per 10 min at 36 C and 27 C, respectively). This finding is not at variance with the data for net photosynthesis and storage in Experiments IV and V, which indicate no change in translocation rate with temperature, if the increase in flow of the ^{14}C tracer through the leaf with increasing temperature, reflects an increase in the percentage of ^{14}C that is transported relative to that which is stored, and also a reduction in the size of the carbohydrate pool through which the tracer must pass.

DISCUSSION

In contrast to the generally accepted view (1, 10, 13), we obtained no evidence that 30 C is critical for the development of systemic symptoms of TMV in *N. glutinosa;* our data showed that systemic symptoms developed in plants exposed to a wide range of temperature; i.e., from 33/28 to 21/16 C. Further, we obtained no evidence that 30 C is in any way critical for the rate of movement of carbohydrate out of a leaf. Although high temperatures promoted the development of systemic symptoms, they had little effect on the rate of translocation of carbohydrate, which was relatively constant over the temperature range of 24 or 27 C to 36 C; i.e., there was no evidence that the promotion of systemic symptoms by high temperatures was associated with an increase in the rate of translocation of carbohydrate out of a leaf.

There appear to be no previous detailed studies on the movement of assimilates in plants which have been used in the study of long distance movement of virus. Our data on physiological processes contributing to movement of assimilates in *N. glutinosa* are within the range of those obtained for the same processes in other plants. For example, the data for net photosynthesis are similar to those for wheat in that net photosynthesis was reduced at high temperatures (14); however, they include lower optima than those found for some plants [e.g., soybean and sugar beet (7)].

Since we have shown that long-distance movement of TMV in *N. glutinosa* occurs in the phloem (4), and since observations made in the present experiments as well as in other reports (1, 13) show that local lesions formed at about 30 C are relatively large and numerous, we suggest that the promotion of the development of systemic symptoms by high temperatures was due, primarily, to relatively large numbers of virus particles reaching the phloem and to the subsequent relatively rapid rate of multiplication in the sink tissues. We do not think it was due to an increase in the speed of movement of virus within the phloem, because the time required for TMV to move long distances in various hosts grown under a range of temperature conditions (4), is much shorter than that

required for the development of systemic symptoms (Fig. 1).

Factors other than relatively high temperature which have been found to promote the development of systemic symptoms are, a relatively high level of inoculum (9), and a continuing supply of virus from the inoculated leaves (15, 16; and Helms, *unpublished*). In the present experiments, both partial defoliation of plants before inoculation and jet-injection of virus into the midvein of leaves promoted the development of systemic symptoms at low temperatures. We suggest that partial defoliation promoted the development of systemic symptoms by enhancing the channeling of virus (as well as carbohydrate) from the single source leaf to the single major sink, the roots. Since parallel work (4) has provided no evidence that long-distance movement of jet-injected virus can occur without prior multiplication, we suggest that jet-injection promoted the development of systemic symptoms by causing virus to multiply in cells which were in close proximity to the main vein of the leaf. The probability of virus entering the sieve tubes of petioles and stems from these cells could be greater than from cells in the leaf lamina which was inoculated by the standard method of mechanical inoculation by leaf rubbing.

LITERATURE CITED

1. BAWDEN, F. C. 1964. Plant viruses and virus diseases. Ronald Press, New York. 361 p.
2. BENNETT, C. W. 1940. Relation of food translocation to movement of virus of tobacco mosaic. J. Agric. Res. 60:361-390.
3. BENNETT, C. W. 1956. Biological relations of plant viruses. Annu. Rev. Plant Physiol. 7:143-170.
4. HELMS, K., and I. F. WARDLAW. 1976. Movement of viruses in plants: long distance movement of tobacco mosaic virus in Nicotiana glutinosa. Pages 283-293. *in* I. F. Wardlaw, and J. B. Passioura, eds. Transport and transfer processes in plants. Academic Press, New York. 484 p.
5. HEWITT, E. J. 1966. Sand and water culture methods used in the study of plant nutrition. Tech. Commun. 22. (Revised 2nd ed.). Commonwealth Bureau of Hort. and Plantation Crops, East Malling, Maidstone, Kent, England. 547 p.
6. HODGKINSON, K. C. 1974. Influence of partial defoliation on photosynthesis, photorespiration and transpiration by lucerne leaves of different ages. Aust. J. Plant Physiol. 1:561-578.
7. HOFSTRA, G., and J. D. HESKETH. 1969. Effects of temperature on the gas exchange of leaves in the light and dark. Planta (Berl.) 85:228-237.
8. HOFSTRA, G., and C. D. NELSON. 1969. The translocation of photosynthetically assimilated ^{14}C in corn. Can. J. Bot. 47:1435-1442.
9. HOLMES, F. O. 1932. Movement of mosaic virus from primary leaves in Nicotiana tabacum L. Contrib. Boyce Thompson Inst. 4:297-322.
10. MC KINNEY, H. H., and E. E. CLAYTON. 1945. Genotype and temperature in relation to symptoms caused in Nicotiana by the mosaic virus. J. Hered. 36:323-331.
11. MORSE, R. N., and L. T. EVANS. 1962. Design and development of CERES – an Australian Phytotron. J. Agric. Eng. Res. 7:128-140.
12. MUMFORD, D. L. 1972. A new method of mechanically transmitting curly top virus. Phytopathology 62:1217-1218.
13. SAMUEL, G. 1931. Some experiments on inoculating methods with plant viruses, and on local lesions. Ann. Appl. Biol. 18:494-507.
14. WARDLAW, I. F. 1974. Temperature control of translocation. Pages 533-538 *in* R. L. Bieleski, A. R. Ferguson, and M. M. Cresswell, eds. Mechanisms of regulation of plant growth. N. Z. Roy. Soc. Bull. 12. 934 p.
15. WEINTRAUB, M., W. G. KEMP, and H. W. J. RAGETLI. 1961. Some observations on hypersensitivity to plant viruses. Phytopathology 51:290-293.
16. WEINTRAUB, M., W. G. KEMP, and H. W. J. RAGETLI. 1963. Conditions for systemic invasion by virus of a hypersensitive host. Phytopathology 53:618.
17. ZAITLIN, M. 1962. Graft transmissibility of a systemic virus infection to a hypersensitive host – an interpretation. Phytopathology 52:1222-1223.