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Long Distance Movement of Cauliflower Mosaic Virus in Infected Turnip Plants

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The long distance movement of cauliflower mosaic virus (CaMV) in systemically infected turnip plants was visualized by leaf blot hybridization techniques. Girdling experiments demonstrated that CaMV moved systemically in turnip plants through phloem channels. In time course experiments, CaMV exited the inoculated leaf and invaded the vasculature five days after inoculation. At this time, foci of viral DNA, as detected by hybridization, first appeared on the inoculated leaf. The movement of CaMV was compared to the translocation of photoassimilates labeled with $^{14}CO_2$. The patterns were similar and influenced by both plant phyllotaxis and leaf developmental stage. Both virus and photoassimilate generally accumulated in younger (sink) leaves on the side of the leaf nearest the point of insertion of the mature inoculated (source) leaf. As leaves underwent the sink-to-source transition in photoassimilate import, there was a progressive basipetal (leaf tip to base) decline in the amount of photoassimilate and the number of virus particles entering the lamina so that, in more mature sink leaves, only the base of the leaf became infected. Therefore, phyllotaxis determines what side of a leaf the virus will invade, and the leaf developmental stage determines how far toward the apex the virus will progress.

Additional keywords: caulimoviruses, systemic movement.

In systemic infections, plant viruses move short distances from cell to cell via plasmodesmata and long distances via the plant vascular system (Agrios 1988; Hull 1989). Viruses appear to invade the vascular system through prescribed pathways because they preferentially follow certain routes in the course of an infection. In a classic study, Samuel (1934) described the pattern of systemic movement of tobacco mosaic virus (TMV) in tomato and tobacco plants.

During long distance movement, viruses encounter many cell types. In certain virus-host combinations, the movement of virus can be blocked at different points. For example, in resistant pepper lines, cucumber mosaic virus (CMV) does not efficiently invade the phloem channels where it normally moves in susceptible plants. Yet in these lines CMV multiplies and spreads from cell to cell within inoculated leaves as well as in susceptible varieties of peppers (Dufour *et al.* 1989). When CMV does invade the vascular system in resistant lines, virus is restricted to one or two phloem bundles in the leaf petiole. CMV was almost never found in the shoot in resistant lines. In the roots, CMV was confined to the vascular bundles it had entered in the leaves. Thus, in resistant peppers, CMV infrequently invades phloem channels leading downward to the root and is almost totally blocked from moving upward.

Most plant viruses are thought to move through the vascular system via phloem. If this is the case, then virus movement should be governed by the same parameters as photoassimilate movement, such as phyllotactic pattern (Turgeon and Webb 1973). Also, transported virus should accumulate in the base, but not the tip, of a growing leaf that is in transition from sink (importer of photoassimilate) to source status (exporter of photoassimilate), because the sink-to-source transition is a developmental event that progresses basipetally in each leaf (Turgeon 1989).

Several types of plant viruses have been observed by electron microscopy in the phloem of infected plants (e.g., TMV [Esau and Cronshaw 1967]), and certain geminiviruses are phloem-limited (Hull 1989). However, several beetle-transmitted viruses move systemically through the xylem (Hull 1989). Aphid-transmitted viruses usually move through the phloem, where aphids feed. An exception is blueberry shoestring virus, which is aphid-transmitted but moves through xylem as well as phloem tissue (Urban *et al.* 1989). The observation that virus particles have been observed in phloem tissue suggests that viruses move long distances through the phloem as virions (Esau and Cronshaw 1967).

This paper examines the pattern of long distance movement of cauliflower mosaic virus (CaMV), an aphid-transmitted DNA virus, in infected turnip plants. To do so, leaf blot hybridization was performed to localize virus infection and track virus movement. Because it was found that CaMV moves through phloem channels, we determined whether the pattern of CaMV movement was similar to that of the translocation of photoassimilates.

MATERIALS AND METHODS

Plants and virus isolates. CaMV isolate CM4-184 was maintained by serial passage in *Brassica campestris* var. *rapa* L. 'Just Right' (turnips) in a greenhouse. Plants were mechanically inoculated with cell sap prepared by grinding infected leaves in 10 mM potassium acetate (pH 7.2) at 2 ml of buffer per gram of tissue. Celite was added at

4 mg ml^{-1} of cell sap, and 50 μl was rubbed on the mature leaves of 3-week-old plants.

Movement of CaMV out of inoculated turnip leaves. The distal halves (avoiding midribs) of the fifth leaf of 3-week-old turnips were mechanically inoculated with cell sap prepared as above. Inoculated leaves were removed at 0–17 days post inoculation (DPI). The uninoculated leaves were periodically examined for systemic symptoms, and the final results were tabulated at 32 DPI. Control plants were mock inoculated with buffer and Celite. The removed inoculated leaves were stained or hybridized as described below.

Leaf blot hybridization and iodine staining of turnip leaves. Leaf blot hybridization was carried out as described by Melcher *et al.* (1981), except that the leaves were extracted initially with ethanol instead of 2-methoxyethanol, and then treated with 0.5 M NaOH/1.0 M NaCl and 1.0 M Tris-HCl (pH 7.5)/1.5 M NaCl instead of 0.5 M NaOH/1.5 M NaCl and 0.5 M Tris-HCl (pH 7.0)/3.0 M NaCl following the proteinase K step. These modifications were introduced to make the procedure more consistent with standard blotting techniques. The 8-kb insert from the plasmid pLW414 (the cloned genome of the CM4-184 isolate of CaMV [Howell *et al.* 1980]) was labeled with an Amersham Multiprime DNA labeling kit according to the manufacturer's instructions and used as a probe (Amersham Corp., Arlington Heights, IL). After hybridization, leaves were stained with potassium triiodide as described by Holmes (1931). Iodine stains leaf starch a dark purple to black color. Viral lesions appear as clear areas on a dark background.

Girdling experiments. Petioles of the fifth leaf of 3-week-old plants were girdled by rubbing a red hot needle around the middle of the petiole (one leaf per plant). The girdled leaves were inoculated approximately 10 min after girdling with cell sap from virus-infected turnip leaves, and the girdled leaves were supported. Plants were inspected periodically for lesions on both inoculated and uninoculated leaves. For controls, the fifth leaf of ungirdled plants was inoculated.

Photoassimilate transport. The pattern of photoassimilate translocation between source (mature) and sink (immature) leaves was determined by labeling a single attached source leaf with $^{14}CO_2$ (Turgeon 1989). The leaf was enclosed in a polyethylene bag and exposed for 5 min to $^{14}CO_2$ (0.5 MBq), generated inside the barrel of a 50-ml syringe by the addition of excess 80% lactic acid to $Na_2{}^{14}CO_3$ (6.6 MBq·$mmol^{-1}$) and injected into the bag. After translocation for 2 hr, sink leaves were removed, quickly placed between two stainless steel screens to keep them flat, and frozen by covering with powdered dry ice. Throughout the experiment the plant was illuminated by a water-filled 1,000-W metal halide lamp (M1000/C/U metalarc; Sylvania, Danvers, MA) providing 400 μmol·photons·m^{-2}·s^{-1} photosynthetically active radiation (PAR) at the level of the labeled leaf. Frozen leaves were lyophilized (Virtis freeze dryer; Virtis Co., Gardiner, NY) for three days at −30° C (condenser at −60° C) to keep the leaf tissue frozen. Lyophilized leaves were flattened between polished steel plates in a large vise, and the flattened leaves were exposed for 3 days to X-ray film (Hyperfilm-βmax; Amersham). Further details are given in Weisberg *et al.* (1988).

RESULTS

Movement of CaMV out of inoculated leaves. The time required for CaMV to establish a systemic infection in turnip plants was determined by removing inoculated leaves at different times post-inoculation. Uninoculated leaves on the remainder of the plant were examined periodically for up to 32 DPI for systemic symptoms following inoculation. No systemic symptoms were observed when inoculated leaves were removed up to 5 days (Table 1). However, when inoculated leaves were removed at 5 DPI or later, systemic symptoms were observed at 21 DPI. Therefore, under our conditions, about 5 days are required for sufficient numbers of infectious particles to exit from inoculated leaves to establish a systemic infection. This agrees with the findings of Melcher (1989) who reported that inoculated leaves had to remain on the plant for 4 or more days for turnips to develop systemic symptoms.

To determine the extent to which virus infection had progressed in the inoculated leaf at the time when systemic infection occurred, we looked for the appearance of viral lesions in inoculated leaves with either iodine staining to

Table 1. Time course of appearance of symptoms and establishment of systemic infection in cauliflower mosaic virus-infected turnip leaves

Days post-inoculation leaf removed	Systemic symptoms after leaf removed[a]	Visible symptoms (chlorotic lesions) on inoculated leaf	Starch lesions on inoculated leaf	Viral foci detectable by hybridization
0	−	−	−	−
1	−	−	−	ND[b]
3	−	−	−	ND
5	+	−	−	+
7	+	+	+	+
9	+	+	+	ND
11	+	+	+	ND
13	+	+	+	+
15	+	+	+	ND
17	+	+	+	ND

[a] Systemic symptoms, visible on the rest of the plant after leaf was removed, were recorded at 32 days post-inoculation. Other symptoms (next three columns) were recorded on the day of leaf removal.
[b] Not determined.

detect starch lesions or by hybridization with CaMV DNA (Melcher *et al.* 1981). Visible symptoms (i.e., chlorotic lesions in the inoculated leaf) were generally not visible until about 7 DPI (Table 1). Starch lesions also did not appear until about that time.

Using the more sensitive hybridization procedure, we found that foci hybridizing to viral DNA appeared by day 5 just before the first visible lesions (day 7) (Fig. 1). The foci were very small and faint, but distinct. More intense hybridization to viral foci was observed in the inoculated leaves 7 and 13 DPI. At 7 DPI, the foci were circular and fairly uniform in size, but varied in intensity of hybridization. At day 13, the DNA probe hybridized to irregularly shaped lesions that varied somewhat in size and in intensity of hybridization. Many of the lesions on the day-13 leaves hybridized more intensely at the periphery of the lesion than in the center. A similar result was reported by Melcher *et al.* (1981). In a few lesions on day-13 leaves, hybridization was observed in the region of the vein directly adjacent to the viral lesion. Therefore, infectious particles in numbers sufficient to produce a systemic infection moved out of the inoculated leaf before visible symptoms or starch lesions appeared, but at about the time when foci hybridizing to viral DNA could first be detected.

CaMV moves through phloem. To determine whether CaMV moves through the phloem or xylem, girdling experiments were performed. Girdling blocks conduction through phloem tissue while leaving the xylem intact. TMV, which moves long distances only through the phloem, cannot move through a girdled stem (Caldwell 1930). On the other hand, blueberry shoestring virus, which can move through xylem as well as phloem tissue, can move through a girdled stem (Urban *et al.* 1989).

Because turnip plants lack an elongated main stem, other than the flower stalk, the petioles of inoculated leaves were girdled. Girdled leaves were inoculated with CaMV, and the plants were monitored for subsequent symptom production. Viral lesions appeared on the inoculated leaves at the same time (7 DPI) in both girdled and ungirdled leaves, and the lesions were similar in size. The girdled leaves remained turgid until about 9 days after girdling, showing that xylem transport was unimpaired at least up to this time. In two ungirdled control plants, systemic symptoms were first observed at 15 DPI and, by 26 DPI, systemic symptoms were visible on about seven leaves per plant. In the four plants with girdled petioles, no visible systemic symptoms appeared at any time. The inability of CaMV to move through a girdled petiole indicates that this virus moves long distances through the phloem.

Patterns of virus distribution relate to phyllotactic positions of leaves. Viral symptoms in systemically infected plants appear in the inoculated leaves and in recently emerged leaves. In leaves that were very immature (less than 0.5 cm. long) at the time virus invaded the vasculature (5 DPI), the entire lamina became uniformly symptomatic, observed by 21 DPI. In older leaves, symptoms often appeared more strongly on one side of the lamina than on the other. The bilateral asymmetry appeared more striking when the distribution of viral DNA was visualized by leaf blot hybridization. An example is shown (Fig. 2A) in which there is extensive hybridization on one side of the leaf with scattered foci on the other. Melcher (1989) also reported that the pattern of lesions in systemically infected leaves was often biased toward one half of the leaf or the other.

This pattern of viral symptoms on various leaves was examined in the context of their phyllotactic position on the systemically infected plant. One leaf on a 4-week-old plant was inoculated, and at 21 DPI all the leaves on the plant were noted for phyllotactic position, removed, and hybridized to the viral probe. Leaf 0 (the inoculated leaf)

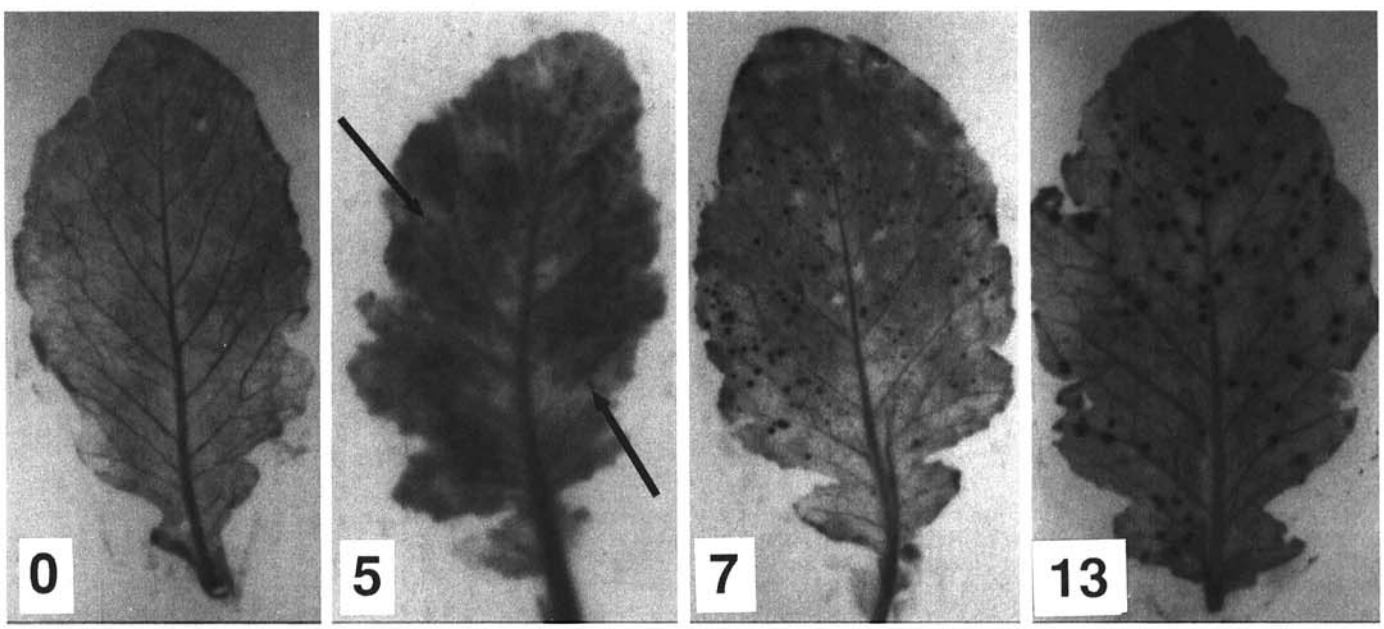

Fig. 1. Time course of appearance of viral foci on turnip leaves inoculated with cauliflower mosaic virus (CaMV). Viral DNA was visualized using a leaf blot hybridization procedure and a ^{32}P-labeled CaMV DNA probe. Leaves inoculated with CaMV were harvested at 0, 5, 7, and 13 days post-inoculation (DPI) and hybridized. Foci of infection for the 5 DPI leaf are indicated by arrows.

showed individual viral lesions, and the pattern of foci of hybridization (Fig. 3) corresponded to the pattern of visible lesions. Leaves 1–4 showed no viral lesions and only background hybridization to the viral probe. Leaf 5 showed only a few hybridizing foci near the basal end of the leaf closest to the inoculated leaf, and these corresponded to visible lesions. Leaves 6, 7, 8, and 10 had visible lesions and foci of hybridization. In leaves 6 and 7, which were on the opposite side of the plant from the inoculated leaf, the foci of hybridization were observed on the side of the lamina closest to the point of insertion of the inoculated leaf. In leaf 8, and to a lesser extent, leaf 10, which were almost directly above the inoculated leaf, foci of hybridization were distributed uniformly over the surface. Leaf 9 possessed a small number of isolated viral lesions on the side of the leaf closest to the inoculated leaf, however visible lesions were not observed. The leaf halves that hybridized most intensely with the viral probe and that were most symptomatic were closest to the point of insertion of the inoculated leaf. Thus, the asymmetrical distribution of virus and viral lesions was influenced by phyllotaxis (Fig. 3.; Fig 4A).

The influence of phyllotaxis on the distribution of virus in infected leaves may be a consequence of the flow of photoassimilates and the pattern of phloem channels that interconnect the inoculated leaf with the systemically infected leaves. This possibility was tested by following the flow of photoassimilates from a source leaf to several sink leaves. (For technical reasons the photoassimilate and virus transport experiments could not be carried out in the same plant.) In these experiments, a source leaf was labeled with $^{14}CO_2$, the sink leaves were noted for their phyllotactic position and analyzed for the distribution of photoassimilates. The sink leaves into which the radioactive photoassimilate was transported were more heavily labeled on the side of the leaves nearest to the source leaf (Fig. 2B; Fig. 4B). Thus, with relation to phyllotaxis, photoassimilates accumulate in sink leaves on the side of the leaf closest to the point of insertion of the source leaf (Fig. 4B). The asymmetric pattern of photoassimilate accumulation in the sink leaf was strikingly similar to the pattern of viral lesions. We also noted that in certain sink leaves, photoassimilate was transported to the opposite side of the leaf at a point about one third the distance to the leaf apex (Fig. 2B). This is an unusual translocation pattern and may be due to anastomosis of vascular bundles in the upper region of the midrib. A similar pattern of virus infection was seen in systemically infected leaves (leaf 7 in Fig. 3 and Fig. 4A).

Patterns of virus distribution correlate with developmental stages of leaves. In addition to the asymmetric distribution of symptoms across the midrib axis, another pattern was observed. Less recently emerged leaves showed a basipetal pattern of symptoms (i.e., more lesions appeared toward the base of the leaf than toward the apex). Generally, in older leaves, virus and viral lesions were confined to the leaf base. The relationship between the basipetal distribution of virus with the developmental state of the leaf was examined in greater detail. A single leaf was inoculated, the lengths of the leaves were determined at 5 DPI, and at 21 DPI the leaves were removed for hybridization to the viral probe. The leaf length at 5 DPI was used as an indicator of developmental state of the various leaves at the time CaMV first exited the inoculated leaf. It was not until 21 DPI, however, that the extent of the leaf surface

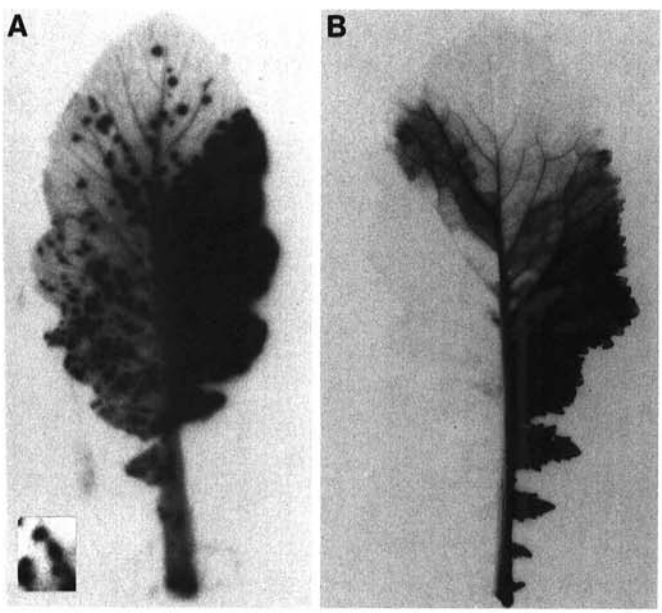

Fig. 2. Comparison of the pattern of accumulation of **A,** CaMV in a systemically infected leaf and **B,** ^{14}C-labeled photoassimilates in a sink leaf. In virus-infected plant (A), the fifth leaf from the base was inoculated with cauliflower mosaic virus (CaMV), and the seventh leaf was prepared for hybridization. The leaf was harvested at 21 days post-inoculation and hybridized to a CaMV probe. In the plant used for photoassimilation experiment (B), the sixth leaf was labeled with $^{14}CO_2$ for 5 min, and 2 hr later the eighth leaf was prepared for autoradiography.

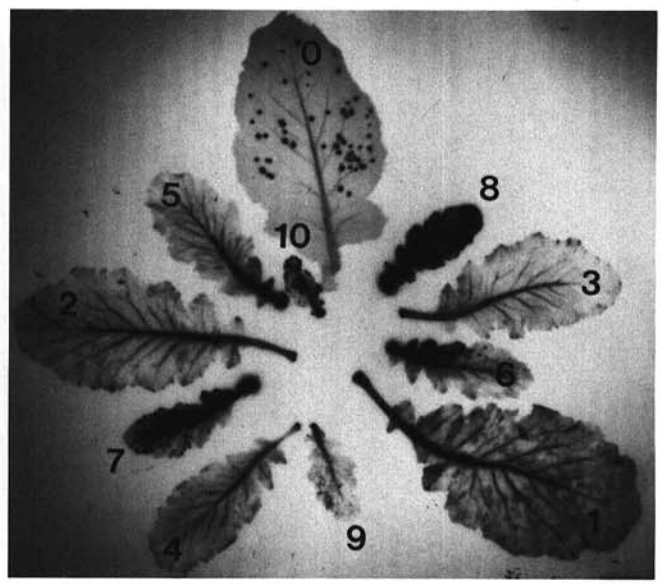

Fig. 3. Phyllotactic arrangement of leaves from a systemically infected turnip plant. Leaf numbered 0 was inoculated with cauliflower mosaic virus (CaMV). Younger leaves were numbered sequentially. Leaves were harvested at 21 days post-inoculation, hybridized to a CaMV probe, and arranged in the phyllotactic pattern for autoradiography.

invaded by virus at 5 DPI could be determined by leaf blot hybridization. The fraction of leaf accumulating virus at a given time = t (in this case 5 DPI), was determined by dividing the length of the leaf hybridizing to the viral probe by the total length of the leaf. By dividing the length of the leaf at 5 DPI by the length of a mature leaf, the fractional leaf length at time = t was obtained. When the fraction of the leaf accumulating virus was plotted versus the fractional leaf length at 5 DPI (Fig. 5), a basipetal reduction in virus accumulation during leaf development was observed. Young leaves less than one third the length of a mature leaf lost their ability to accumulate virus progressively and basipetally. Leaves larger than about one third the length of a mature leaf did not accumulate and presumably did not import virus at all.

This pattern of virus accumulation and import was compared to the pattern of transport of photoassimilates, because it has been demonstrated that the transition in the sink-to-source transport of photoassimilates also occurs basipetally in leaves during development (Turgeon 1969). These experiments were performed, as described above, by labeling photoassimilates in a source leaf with $^{14}CO_2$ and tracking the movement into younger sink leaves. As expected, the results generally demonstrated that sink leaves into which the radioactive photoassimilate was transported were more heavily labeled at the base than the tip. When the fractional length of the leaf importing photoassimilates was plotted against the developmental state of the leaf (fractional leaf length) at the time of the experiment, it was demonstrated that turnip leaves rapidly lose their ability to import photoassimilates, and they did so in a basipetal fashion (Fig. 5). However, the ability to accumulate photoassimilates declined at a later developmental stage than the ability to accumulate virus, and leaves did not lose the ability to import photoassimilates until they reached about seven tenths of their mature length. Hence, both

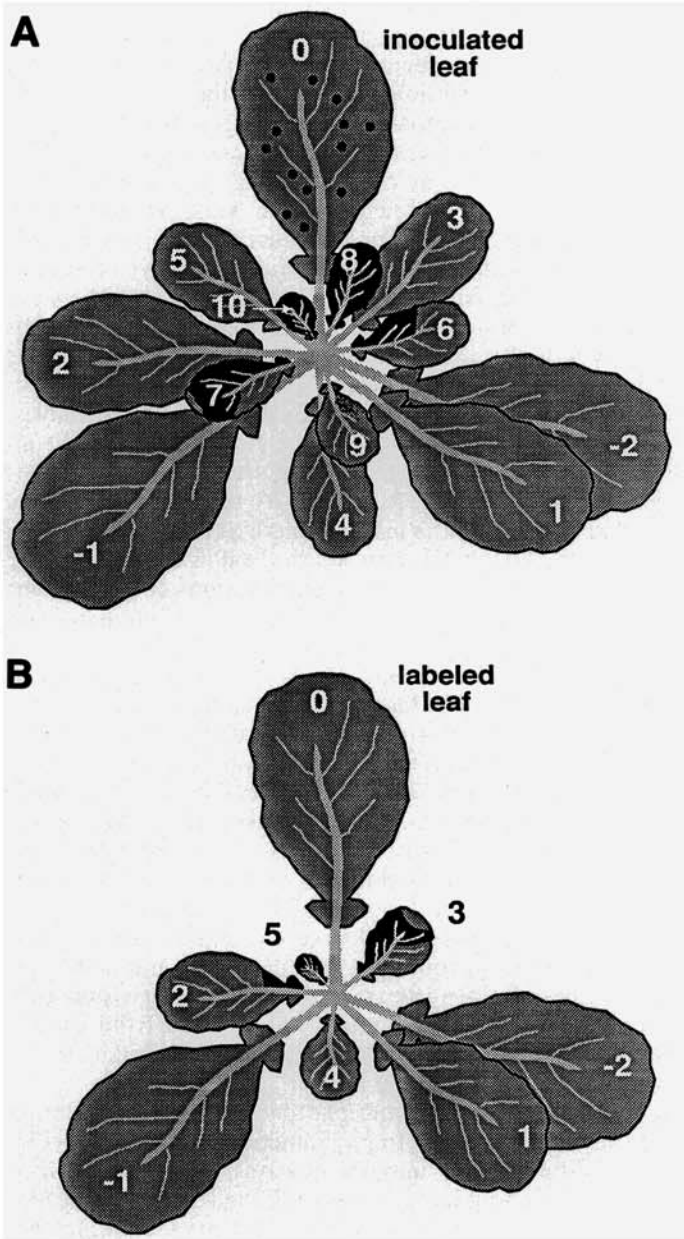

Fig. 4. Diagram comparing movement of **A,** cauliflower mosaic virus (CaMV) in a systemically infected plant and **B,** ^{14}C-labeled photoassimilates from source to sink leaves. Inoculated leaf or source leaf (labeled leaf) is indicated by the number 0. Leaves emerging before the inoculated leaf or source leaf were numbered negatively in reverse order. Leaves emerging after the inoculated leaf or source leaf were numbered positively in sequence. Diagram in (A) is derived from the experiment described in Figure 3. Dark shading represents either viral symptoms (A) or photoassimilate (B).

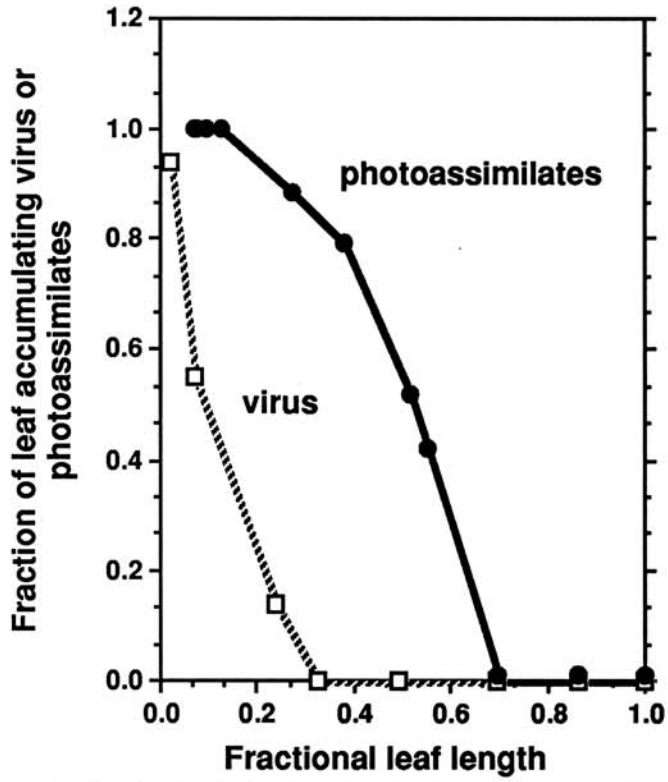

Fig. 5. Plot showing basipetal decline in virus and photoassimilate accumulation in developing turnip leaves. The fraction of leaf length accumulating virus or photoassimilate at 5 DPI is plotted against the fractional length of the leaf at 5 DPI. Data were obtained from measurements of photoassimilate and virus accumulation patterns in autoradiographs of leaves as described in the text.

photoassimilate and virus accumulation decline basipetally in developing turnip leaves, but the ability to accumulate virus diminishes earlier in leaf development than the ability to import photoassimilates.

DISCUSSION

The pattern of CaMV movement in turnip plants is quite predictable. Because CaMV moves through the phloem, movement of the virus is governed by the same parameters that direct the movement of photoassimilates. In particular, the movement of virus is influenced by the developmental stage of the invaded leaves and the phyllotactic relationship between the inoculated leaf and the leaves into which the virus moves. The effect of phyllotaxis on virus and photoassimilate transport presumably reflects the interconnections (or lack thereof) between the vertical phloem bundles through which these substances move upward in the plant (Fig. 6). Young leaves that emerge directly over the insertion point of the inoculated or source leaf uniformly accumulate virus and photoassimilates on both sides of the midrib.

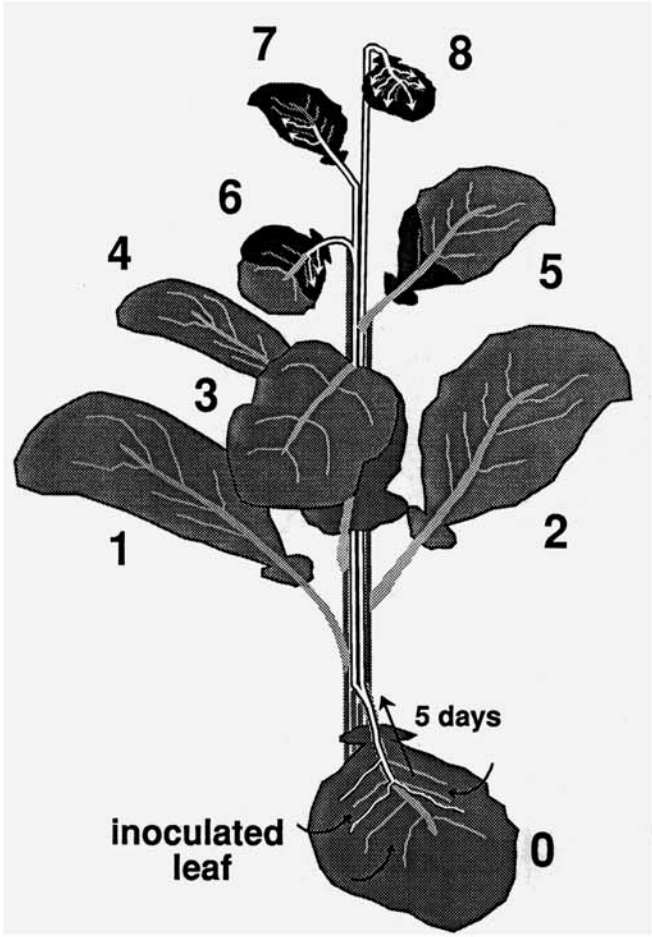

Fig. 6. Exploded diagram of turnip plant to show parameters that influence the bilateral and basipetal accumulation of virus in leaves of systemically infected turnip plant. Inoculated leaf is indicated as leaf number 0. White lines indicate the inferred vascular bundles through which virus moves from inoculated leaf to younger systemically infected leaves. Shading of the leaves indicates the source-to-sink transition of photoassimilate, with the light shading representing the mature (source) part of a leaf and the dark shading representing the immature (sink) part of a leaf.

Young leaves not directly over the insertion point accumulate photoassimilates and virus on the side closest to the insertion point on one side of the midrib, whereas leaves on the opposite side of the plant often fail to accumulate virus or photoassimilates (leaf 9, Fig. 4A; leaf 4, Fig 4B). This pattern of photoassimilate transport is well documented (e.g., Turgeon and Webb 1973). Even though the distribution of photoassimilates is asymmetrical with respect to phloem transport, it is not completely so. Recently emerged leaves are such strong sinks that uneven transport to one side appears to produce uniform symptoms over the leaf. Finally, the apparent anastomosis between the veins supplying the side of the leaf closest to the insertion site and a vein on the other side of the leaf could also help to distribute photoassimilates or virus more uniformly.

The movement of virus and photoassimilates into sink leaves is determined by developmental stage and becomes progressively more restricted to the basal portion of the lamina during growth. For photoassimilates, this process is called the sink-to-source transition and may be due to the closing of plasmodesmatal channels that join the sieve element-companion cell complex to surrounding cells (Turgeon 1989). The loss in ability to accumulate virus follows the same general trend as the basipetal decline in the ability to import photoassimilates in developing leaves. The decline in virus accumulation, however, occurs at an earlier stage in development than the decline in photoassimilate uptake (Fig. 5; Fig. 6). By the time the leaf has reached one third of its mature length, it no longer imports virus even though the base of the leaf is still a sink for photoassimilates. At least two explanations could account for the difference between the decline in photoassimilate and virus uptake. First, there may be a higher threshold for the production of viral lesions than for the import of photoassimilates. The loss of import capacity occurs gradually during the sink-to-source transition so that the base of the lamina imports much more photoassimilate than the apex (Turgeon 1987). It is possible, therefore, that virus would not be imported into the tissue near the sink-to-source boundary because that part of the leaf would not strongly import photoassimilates or the virus particles dispersed in the phloem sap. Second, if the diameter of plasmodesmata connecting the sieve element-companion cell complex to the surrounding cells decreases gradually preceding the sink-to-source transition, then it is possible that the larger virus particles might be excluded from certain parts of a leaf into which the smaller photoassimilate molecules would still be able to move.

The patterns of virus and photoassimilate accumulation are similar with respect to phyllotactic considerations (Fig. 4) but differ in two important ways. First, as described above, developing leaves between 30 and 70% of their mature length import photoassimilates but not CaMV. Leaves at 30% of their mature length can no longer import virus but can import photoassimilates almost to their apices. Second, because it takes about 5 days for virus to exit the inoculated leaf following inoculation, the parameters that determine the systemic movement of the virus come into play following a lag. Under our conditions, the lag is about 5 days or two plastochrons (i.e., the emergence of two new leaves). Thus, the pattern of virus accumulation

in the plant changes after the time of inoculation by about two plastochrons. A leaf that is capable of importing virus at the time of inoculation, may lose that ability by the time the virus becomes systemic. This can be seen by comparing Fig. 4A and B. In Fig. 4B, leaf 5 is still young enough to be a major importer of photoassimilates, but if a plant of this age is inoculated with virus, leaf 5 does not become infected (Fig. 4A). In general, the pattern of viral symptoms in a systemically infected leaf is affected by two major factors: 1) the phyllotactic relationship of inoculated and systemic leaves determines on what side of the midrib the lamina will become infected, and 2) the developmental stage of a systemic leaf at the time of invasion determines how far toward the tip the virus will progress.

The lag in virus movement from an inoculated leaf suggests that virus does not immediately enter the vascular system upon inoculation. Current thinking about the way in which viruses produce a systemic infection from an inoculated leaf is that they do not enter the vasculature directly from the inoculation site. Instead, viruses replicate and move locally until they gain access to the vascular system (Agrios 1988). Movement into the vascular system through several different cell types appears to be slower than cell-to-cell movement through mesophyll cells. For example, movement of other viruses, such as TMV, into the vascular system takes appreciably longer (4–5 days) than movement through the mesophyll (1–2 days) (Schneider 1965). Another interpretation of the lag phenomenon is that 5 days may be required for the virus to reach sufficient titer to produce a systemic infection. Alternatively, the lag may result from time required for the expression of a viral gene product required for long distance movement.

Lesions that formed on inoculated leaves at 7 and 13 DPI were usually associated with the leaf lamina rather than with the major veins, even though virus was inoculated evenly over the entire leaf surface. Many lesions were found close to major veins but were generally not located directly over them. The pattern of viral lesions at 7–13 DPI with respect to the vasculature in inoculated leaves suggests that CaMV infects cells of the lamina and invades the leaf vascular system through minor veins. No hybridization was observed in the veins of leaves at 7 DPI. This observation would argue that CaMV does not necessarily replicate in the vascular system of the inoculated leaf but can move through vascular tissue without initiating detectable foci of infection. This agrees with the work of Samuel (1934) who found that TMV could traverse regions of the stem of tomato plants without initiating an infection. Nonetheless, hybridization was observed in leaf veins (or in the cells surrounding veins) harvested 13 DPI, which showed that CaMV can initiate infections in veins if given enough time. Hybridization in veins was always observed immediately adjacent to viral lesions.

Nodular lesions were observed along major veins of systemically infected leaves (Fig. 2A, insert). These lesions might represent the pathway taken by CaMV when it exits a vein. It appears that the virus exits the vascular bundle and invades the parenchyma at some point along the vein. As the virus moves through the parenchyma, the lesion expands and takes on the morphology of a nodule along the major vein. Nodular shaped lesions were spaced out along the veins, indicating that the virus exits the vein somewhat infrequently.

ACKNOWLEDGMENTS

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