# Translocation of Tobacco Ringspot Virus in Soybean

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## ABSTRACT

Tobacco ringspot virus (TRSV) moved from top leaves to roots of young soybean in 2 to 3 days, but upward translocation of TRSV from roots or lower stem occurred only under certain conditions. Soybean seedlings inoculated below the first node became systemically infected only if inoculated before they were 12 days old. Inoculation between the first and second nodes gave similar results except that some systemic infections occurred when plants were 17 days old at inoculation. The number of plants that became systemically infected increased with time after inoculation at a given age.

Upward translocation of TRSV was slowed at the first and second nodes in soybean seedlings. Major vascular bundle traces in the lower stem of seedlings terminated at the first or second nodes. Additional traces which were continuous through both nodes developed as the plant grew.

In older plants, the major direction of virus movement in the stem below the midpoint of the plant was downward; whereas, virus was translocated both upward and downward when inoculated above the midpoint of the plant. There was no upward or downward movement of TRSV in xylem.

Sieve tubes in the stem below the second node of systemically infected plants, which had been inoculated below the first node, contained crystals and aggregates of TRSV, and aggregates also occurred in companion cells, vascular parenchyma cells, and bundle sheath cells. Virus particles in the plasmodesmata, enlarged plasmodesmata containing clumps of virus particles, and cell wall protrusions enclosing tubules containing virus particles were also observed in these cells.

We suggest that cell-to-cell movement of TRSV from inoculated areas to phloem sieve tubes, and long distance translocation of the virus in the phloem, cause systemic infection of soybean with TRSV.

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Tobacco ringspot virus (TRSV) becomes systemic in seedlings of a number of plants, including soybean, within 3-10 days after mechanical inoculation of cotyledons or leaves (3, 8, 12, 24). However, systemic invasion of plants following root inoculations varies in different plant species (3, 8, 12, 16). Since transmission of TRSV by the nematode *Xiphinema americanum* is to roots (15), its effectiveness as a vector of the virus to various plant species would be related to subsequent upward movement of the virus in these plants.

In cucumber, top symptoms developed within 10 days to 3 weeks in most plants after nematode transmission (15). Moore & McGuire (16) found no difference in time of systemic invasion of the root systems of zinnia and cucumber following point inoculations of roots, mechanically or by nematodes, but translocation of the virus to top leaves of zinnia occurred infrequently or more slowly than in cucumber. TRSV moved upward from the point of mechanical root inoculation very slowly in tobacco and tomato (12), and Bergeson et al. (3) reported limited movement of TRSV from roots to top leaves in soybean, although virus moved from roots to stems in some plants in 4 days.

Preliminary tests indicated that time required for systemic infection of soybean or zinnia with TRSV did not differ following inoculation of roots vs. inoculation of stems below the first node. Therefore, this study was made to determine the patterns of translocation of TRSV in soybean, the tissues in which translocation occurs, and some factors influencing upward translocation from below the cotyledonary node and primary leaf node to top leaves in soybean.

MATERIALS AND METHODS.—*Virus.*—A watermelon isolate of TRSV (PV-125, ATCC), serologically identical to Gooding's NC-72 strain (13) and transmitted by *Xiphinema americanum* (15), was used in all tests. The virus was purified from infected cucumber (21), standardized at ca. 1.5 mg/ml of 0.05 M phosphate buffer, pH 7.0, and frozen. Inocula were prepared by thawing and diluting the suspension to the desired concentration with the same buffer.

*Inoculation procedures.*—Primary leaves of 7- to 10-day-old soybean seedlings [*Glycine max* (L.) Merrill 'Hill' or 'Lee'], or trifoliolate leaves of older plants, were inoculated with TRSV by rubbing with a gauze pad soaked in a 1:10 dilution of the standard virus suspension. In initial tests, soybean stems were inoculated below the first (cotyledonary) node or the second (primary leaf) node by scratching and pricking the stem with a No. 0 dental root-canal file beneath a drop of a 1:5 dilution of standard purified TRSV suspension. In later tests, stems inoculated in this manner were also dusted with Carborundum and rubbed with a cheesecloth pad soaked in a 1:3 dilution of standard virus suspension. Plants were inoculated when they were 7 to 10 days old unless otherwise indicated.

*Indexing procedure.*—Portions of soybean stems or leaves were indexed for presence of TRSV by grinding in 0.05 M phosphate buffer, pH 7.0, and inoculating the primary leaves of cowpea [*Vigna sinensis* (Torner) Savi 'Monarch' or 'Early Ramshorn'] and the cotyledons of cucumber (*Cucumis sativis* L. 'Model'). In most tests, three portions of each plant were indexed: (i) the stem below the first node, (ii) the stem between the first and second nodes, and (iii) top leaves (any young

TABLE 1. Recovery of tobacco ringspot virus from various parts of soybean following inoculation of plants having eight to 10 nodes

| Inoculation of leaf at node no.[a] | No. plants infected | Days after inoculation when virus was recovered[b] | | |
|---|---|---|---|---|
| | | Roots | Leaf at 4th-5th node | Top leaf at 10th-14th node |
| 8-10 | 10 | 7-10 | 5-10 | 7-10 |
| 5 | 4 | 7-17 | 7-14 | 13[c] |
| 4 | 4 | 7-10 | 0[d] | 0[d] |
| 3 | 7 | 7-10 | 28[c] | 0[d] |
| 2 | 2 | 10 | 17[c] | 0[d] |

[a] Trifoliolate leaves and emerging buds of secondary trifoliolate branches mechanically inoculated with tobacco ringspot virus.

[b] Portions of roots and youngest trifoliolate leaves at the designated nodes were indexed to cowpea and cucumber at 3- to 5-day intervals after inoculation.

[c] One plant only.

[d] No virus was recovered by indexing up to 28 days after inoculation, which was the last time plants were indexed.

leaves above the fifth node). In some tests, subdivisions of these regions or individual leaves from a given node were indexed. Roots of plants inoculated below the second node were not always indexed, since TRSV was always present in roots of plants that had virus in the stem or in top leaves. TRSV could be recovered from any portion of the plant when it was recovered from top leaves; i.e., the plant was systemically infected. Presence of local necrotic lesions on cowpea, and a systemic chlorotic mottle in cucumber was considered evidence of TRSV in the indexed portion of the soybean plant.

*Virus transport in xylem.*—Two- to 4-week-old soybean plants were inoculated with TRSV on top leaves, or on young leaves near the middle or lower portion of the stem. A 3-cm portion of stem, either above or below the inoculated leaves, was rotated in a jet of steam for ca. 2 min to kill living cells (5, 20). The plants were indexed for virus above and below the steamed area 10 to 28 days later. Fungicides were applied periodically to retard the growth of saprophytic fungi on the dead tissues of the steamed area.

*Stem anatomy.*—Cotyledonary nodes and primary leaf nodes of 2- to 3-week-old soybean plants were fixed in 3% glutaraldehyde in 0.05 M phosphate buffer, pH 6.8, dehydrated in a graded ethanol series, and embedded in glycol methacrylate (10). Cross sections made on a rotary microtome were stained with toluidine blue and acid fuchsin or periodic acid-Schiff stain (PAS) and toluidine blue (10) and examined microscopically.

Portions of soybean stems less than 10 days old, which included the first and second nodes, and the bark (tissues from the vascular cambium outward) of older plants were cleared using a modification of Foster's technique (11) in order to observe the longitudinal arrangement of phloem fibers. The tissue was boiled for 15 min in water, then boiled 15 min in 95% ethanol, and rinsed in water. This was followed by immersion in 10% NaOH for 4 weeks with frequent changes of the NaOH solution. Cleared material was rinsed in distilled water and stained in 50 ml of 50% ethanol by adding five drops of 0.1% safranin.

*Electron microscopy.*—Portions of stems, including first nodes and internodes between the first and second nodes of 20-day-old soybean plants were prepared for electron microscopy. These plants had become systemically infected with TRSV after inoculation below the first node when they were 1 week old. The pieces of stem were fixed for 48 hr at 4 C in 4% gluteraldehyde in 0.05 M cacodylate buffer at pH 7.4 (14). After fixation, the material was washed several times with buffer solution, postfixed in 1% osmium tetroxide for 2 hr at room temperature, and stained for 6 hr in aqueous 0.5% uranyl acetate. The tissue was dehydrated in a graded ethanol series, embedded in Epon 812, and sectioned

TABLE 2. Presence of tobacco ringspot virus in parts of 'Hill' soybean plants following mechanical inoculation of leaves or stems[a]

| Point of inoculation | Weeks after inoculation | Number of plants with TRSV present[b] | | | |
|---|---|---|---|---|---|
| | | Root[c] | Stem below 1st node | Stem between 1st and 2nd node | Top leaves |
| Primary leaf[d] | 3-5 | 15 | 15 | 15 | 15 |
| Stem below 1st node[e] | 4-6 | 66 | 66 | 59 | 11 |
| Stem below 1st node[f] | 4-6 | 57 | 57 | 52 | 34 |
| Stem between 1st and 2nd node[f] | 4-6 | 37 | 37 | 37 | 37 |

[a] Data are composite of several tests.

[b] Presence of TRSV determined by indexing to cowpea and cucumber.

[c] Number also is total plants with TRSV; i.e., virus was recovered from roots of all infected plants.

[d] Inoculated by rubbing with gauze pad soaked in purified virus suspension after dusting with Carborundum.

[e] Inoculated by pricking stem beneath a drop of virus suspension.

[f] Inoculated by rubbing and pricking.

TABLE 3. Effect of time on upward translocation of tobacco ringspot virus in 'Hill' soybean following inoculation of stems below the cotyledonary node

| Test | Weeks after inoculation | Number of plants with TRSV present[a] | | |
|---|---|---|---|---|
| | | Stem below 1st node[b] | Stem between 1st & 2nd node | Top leaves |
| 1[c] | 1 | 8 | 0 | 0 |
| | 2 | 6 | 0 | 0 |
| | 4 | 8 | 8 | 3 |
| | 6 | 7 | 7 | 1 |
| | 8 | 8 | 8 | 1 |
| 2[d] | 1 | [e] | | 3 |
| | 2 | | | 9 |
| | 3 | | | 11 |
| | 4 | | | 14 |
| | 5 | | | 17 |
| | 6 | 43 | 38 | 27 |

[a] Presence of TRSV determined by indexing on cowpea and cucumber.
[b] Number also is total plants with TRSV; i.e., virus was recovered from below the 1st node of all infected plants.
[c] Stems inoculated by pricking beneath a drop of purified virus suspension.
[d] Stems inoculated by pricking beneath a drop of purified virus suspension, then dusted with Carborundum and rubbed with a gauze pad soaked in virus suspension.
[e] Blank indicates plants were not indexed.

with glass knives on an LKB Ultrotome. Sections were stained in 2% uranyl acetate and lead citrate (17), and examined with a Siemens-Elmiskop 1A. Noninoculated material was prepared and examined in the same manner.

RESULTS.—*Translocation of TRSV after leaf inoculation.*—Systemic infection occurred in all soybean plants mechanically inoculated with TRSV on the primary leaf before trifoliolate leaves emerged. Translocation to roots occurred in many plants within 48 to 72 hr after inoculation.

To determine direction of virus translocation in various parts of older soybean plants, trifoliolate leaves of plants with several nodes were inoculated with TRSV. Portions of roots and pieces of leaf tissue from various parts of the plant were indexed at 5-day intervals after inoculation to detect virus translocation. TRSV was recovered within 10 days from the roots of a majority of plants that had been inoculated on top leaves and upward translocation to developing trifoliolates was detected in 5 to 10 days in these plants (Table 1). Plants inoculated on young trifoliolates of branches emerging from the fifth node or below showed no translocation of TRSV to the top leaves in 28 days, except that virus was recovered from top leaves of one plant 13 days after inoculation at the fifth node. Virus was obtained from roots of most of these plants within 7 to 10 days. Translocation upward to the fourth node occurred in one plant inoculated on a branch emerging from the second node and to the fifth node in a plant inoculated at the third node, but virus was not detected above these points (Table 1).

There was no indication of translocation of TRSV in the xylem of soybean. No virus moved upward or downward through a 3-cm steamed segment of stem in 10 to 28 days after inoculation of trifoliolate leaves above or below the steamed region. Virus was recovered only from the inoculated regions.

*Translocation of TRSV after stem inoculations.—1) Inoculation procedure.*—A combination of the pricking and rubbing inoculation methods consistently resulted in 80-100% transmission; whereas, the pricking method alone was inconsistent and varied from 0-95% transmission. There was an increase in the amount of systemic infection when the former method was used (Table 2, 3).

*2) Effect of nodes.*—Upward translocation of TRSV following inoculation of stems was slowed or restricted at the first node, whereas movement downward was not restricted (Table 2, 3, 4, 5). Upward movement was also slowed at the second node (Table 3, 4, 5). When stems of 7- to 10-day-old soybean seedlings were inoculated below the first node, TRSV was present in the roots and the stem below the second node in most plants 4 to 6 weeks later (Table 2), but many of these plants were not systemically infected. All plants inoculated between the first and second nodes were systemically infected 4 to 6 weeks later (Table 2).

*3) Effect of time.*—The amount of upward

TABLE 4. Recovery of tobacco ringspot virus from various segments of soybean plants following stem inoculation below the cotyledonary node[a]

| Weeks after inoculation | Number of plants with TRSV[b] | | | | | |
|---|---|---|---|---|---|---|
| | At point of inoculation | Above inoculation point but below 1st node | Directly above 1st node | Directly below 2nd node | Directly above 2nd node | Top leaves |
| 1 | 7 | 7 | 3 | 2 | [c] | [c] |
| 2 | 5 | 5 | 1 | 1 | 1 | 1 |
| 4 | 6 | 6 | 6 | 5 | 3 | 3 |
| 6 | 6 | 6 | 5 | 5 | 5 | 5 |

[a] Inoculated by rubbing with a guaze pad soaked in purified virus suspension after dusting with Carborundum.
[b] Presence of TRSV determined by indexing on cowpea and cucumber.
[c] Not indexed.

TABLE 5. Effect of age of soybean at time of inoculation on upward translocation of tobacco ringspot virus

| Test | Age in days of plant when inoculated | Number of plants with TRSV after 6 weeks[a] | | |
|---|---|---|---|---|
| | | Stem below 1st node[b] | Stem between 1st and 2nd node | Top leaves |
| A. Stem inoculated below 1st node[c] | 2 | 24 | 24 | 18 |
| | 7 | 31 | 30 | 10 |
| | 12 | 20 | 15 | 0 |
| | 17 | 15 | 11 | 0 |
| | 22 | 13 | 10 | 0 |
| | 28 | 8 | 5 | 0 |
| B. Stems inoculated between 1st and 2nd nodes[c] | 7 | 18 | 18 | 18 |
| | 12 | 22 | 22 | 20 |
| | 17 | 25 | 25 | 10 |
| | 22 | 24 | 24 | 0 |

[a] Presence of TRSV determined by indexing on cowpea and cucumber.
[b] Number also is total plants infected with TRSV; i.e., virus was recovered from below the 1st node of all infected plants.
[c] Inoculated by pricking the stem beneath a drop of purified virus suspension, then rubbing with a gauze pad soaked in virus suspension after dusting the stem with Carborundum.

translocation of TRSV from the inoculated region of soybean stems varied between tests and with inoculation method, but generally increased with time (Table 3). In one test, no virus was recovered above the first node in the first 2 weeks after inoculation below the cotyledonary node, but after 4, 6, and 8 weeks virus was present in the internode above the first node of all infected plants and some plants were systemically infected (Table 3). In a similar test, top leaves of plants were indexed 1, 2, 3, 4, and 5 weeks after inoculation, then portions of the stem as well as the top leaves (any young leaves above the fifth node) of these plants were indexed for presence of TRSV 6 weeks after inoculation. Only three of 43 infected plants were systemically infected after 1 week, as indicated by presence of virus in top leaves, but the number of systemically infected plants increased with time (Table 3).

The rate of upward movement of TRSV from the inoculated region in the stem was examined more closely by indexing several subdivisions of the stem from slightly above the second node down to the point of inoculation 1, 2, 4, and 6 weeks after inoculation. TRSV was recovered from stem tissue between the point of inoculation and the first node of all infected plants at each time interval (Table 4). During the first 2 weeks, virus was recovered above the first node in only one-third of the infected plants; whereas, after 4 to 6 weeks virus had moved through the first node of most plants. In one plant each at 1 week and 4 weeks, virus was recovered from the lower portion of the internode above the first node but not from the upper portion of this internode. The number of systemically infected plants increased with time after inoculation. All plants from which virus was recovered from the stem directly above the second node also yielded virus from top leaves, which is indicative of systemic infection (Table 4).

*4) Effect of age of plant at inoculation.*—The distance TRSV was translocated upward in 6 weeks from the point of stem inoculation of soybean decreased as the age of the plant at the time of inoculation increased (Table 5). When stems of 2-day-old soybean seedlings were inoculated below the first node, virus was recovered 6 weeks later from this region and the internodal region above this node in all plants, and from the top leaves of 75% of the plants. Similar results were obtained from plants inoculated at 7 days of age except that less than 35% were systemically infected. Plants inoculated when they were 12 days old or older were not systemically infected after 6 weeks, and movement of TRSV through the first node had occurred in successively fewer plants as age at inoculation increased (Table 5). Similar results were obtained when plants were inoculated in the internode between the cotyledons and the primary leaves, but a decrease in number of systemically infected plants did not occur until plants were 17 days old at the time of inoculation (Table 5).

*Lower stem anatomy.*—Cross-sections of soybean stems showed that each phloem bundle is surrounded on the outer side by a layer of sclerid fibers or perivascular fibers (9). Older plants generally had thicker layers of perivascular fibers than young soybean seedlings. The cell walls of the perivascular fibers were also thicker in older plants. The sclerids stained dark red with safranin in cleared material, while other tissues stained faintly or not at all. Thus, phloem bundles could be followed in the internodes and nodes of whole stems or bark.

Six prominent phloem bundle traces were present in the stem below the first node in soybean seedlings (Fig. 1-A, 1-B). Four of these phloem traces entered the cotyledons and stopped. Two traces of vascular bundles which were continuous through the first node entered the primary leaves and discontinued there. A third trace which originated below the first node as a branch from a major trace was also continuous through the first node and formed a "T" at the second node to enter both primary leaves (Fig.

1-A, 1-B). It was visible only on one side of the cleared stem. Developing phloem bundles in the first internode of soybean less than 10 days old, anastomosed with the primary leaf traces below the first node, and all but a small portion of two traces were continuous past the second node. As the plants

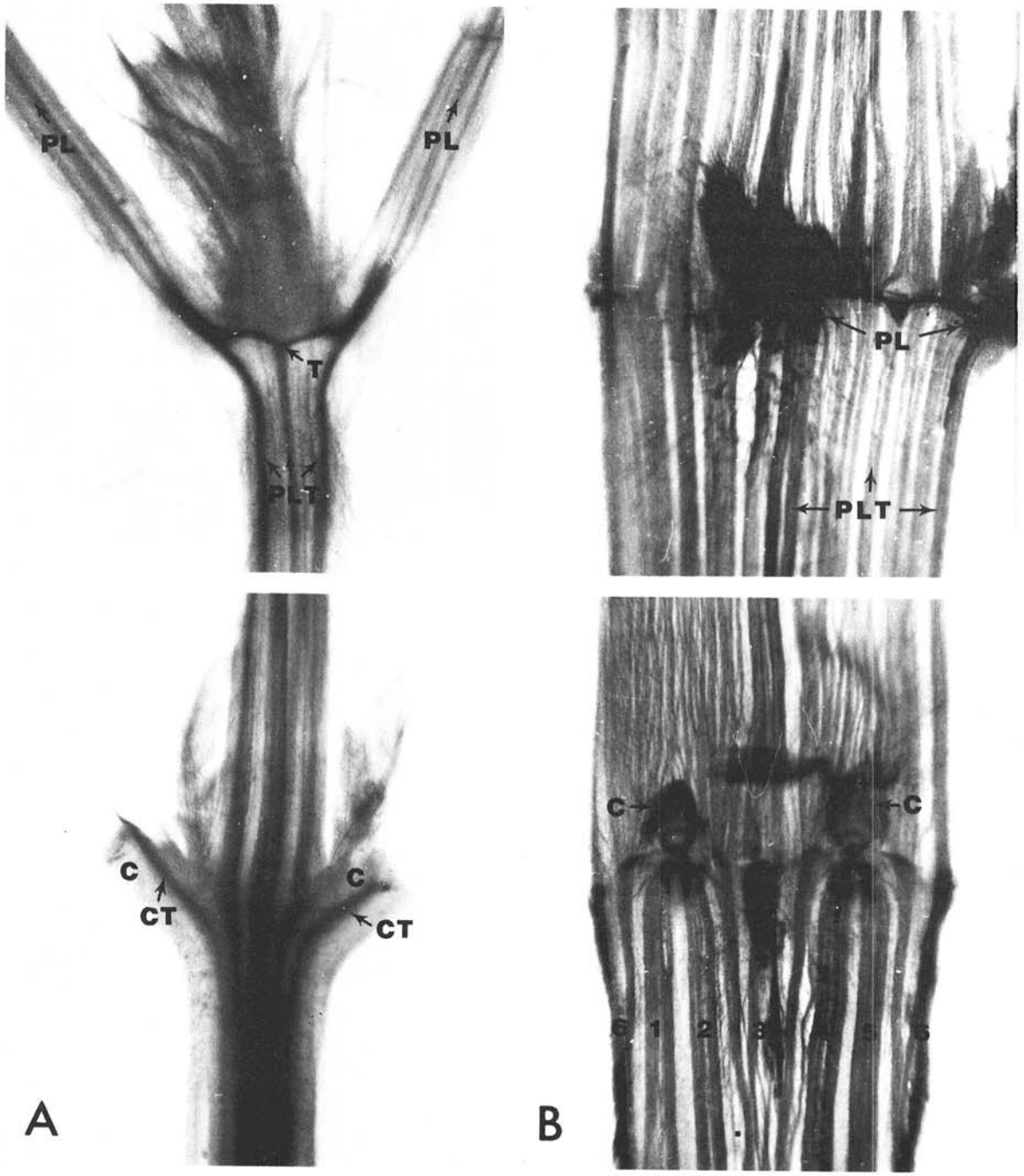


**Fig. 1. A)** Stem of 6-day-old soybean including the first and second nodes; **B)** Pieces of the bark (vascular cambium outward) from 4-week-old soybean stems showing phloem fibers from below the first node to above the second node (×8). Four of six major phloem traces below the first node terminate in the cotyledons and the other two traces terminate in the primary leaves. A third primary leaf trace which originates as a branch from one of the two continuous traces enters both primary leaves via a "T" connection at the second node. In B, ca. 10 other minor traces that also branch from these two major traces are continuous through the second node. C = cotyledons; PL = primary leaf; PLT = primary leaf trace; T = "T" connection.

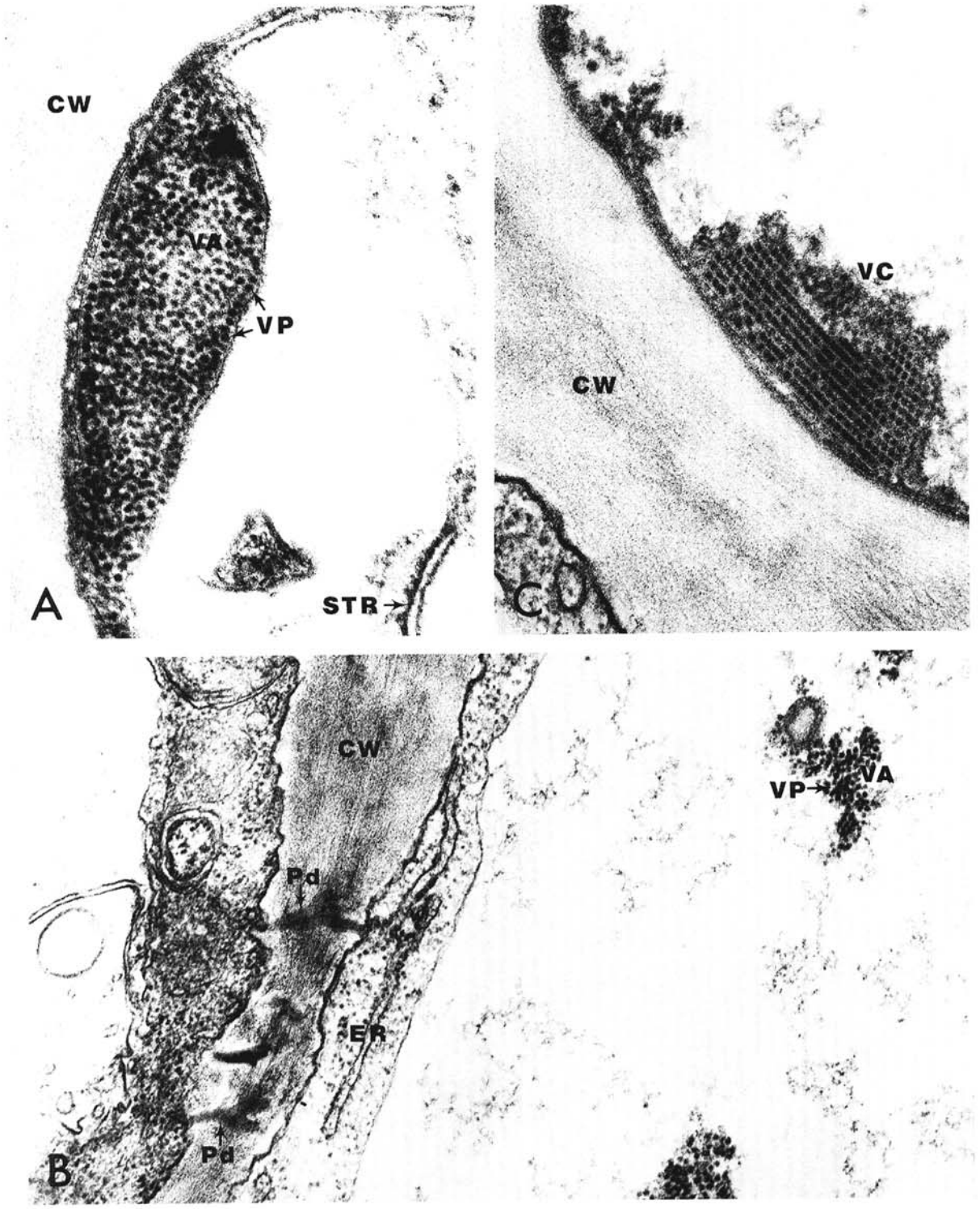


Fig. 2. Cross section through stems of tobacco ringspot virus-infected soybean. **A)** Sieve tube containing an aggregate of virus particles (×75,000); **B)** Bundle sheath cell with aggregates of TRSV in the central vacuole (×40,000). Virus particles are also present in the plasmodesma connecting this cell to a vascular parenchyma cell; **C)** Sieve tube with a crystal of TRSV particles (×75,000). CW = cell wall; ER = endoplasmic reticulum; Pd = plasmodesma; STR = sieve tube reticulum; VA = virus aggregate; VC = virus crystal; VP = virus particles.

matured and trifoliolate leaves formed, their phloem bundle traces which were continuous from below the first node past the second node increased in number and size. These findings are similar to those of Bell (1) and Weaver (23).

*Electron microscopy.*—Aggregates of virus-like particles were observed in phloem sieve tubes (Fig. 2-A) and also in related companion cells and parenchyma cells of the bundle sheath in stems of soybean systemically infected with TRSV (Fig. 2-B). Crystals of TRSV were also found in sieve tubes (Fig. 2-C). Many developing phloem cells were infected, but none of the cambial cells observed contained virus particles. Virus particles were seen in plasmodesmata (Fig. 2-B, 3, 4-A) connecting bundle sheath cells, vascular parenchyma cells, companion cells and sieve tubes. Large clumps of particles were sometimes observed within enlarged plasmodesmata (Fig. 4-A). Virus particles enclosed in tubules inside of cell wall protuberances (Fig. 4-B) were found in bundle sheath cells, vascular parenchyma cells and companion cells.

DISCUSSION.—The slowness of upward translocation of TRSV from below the first and

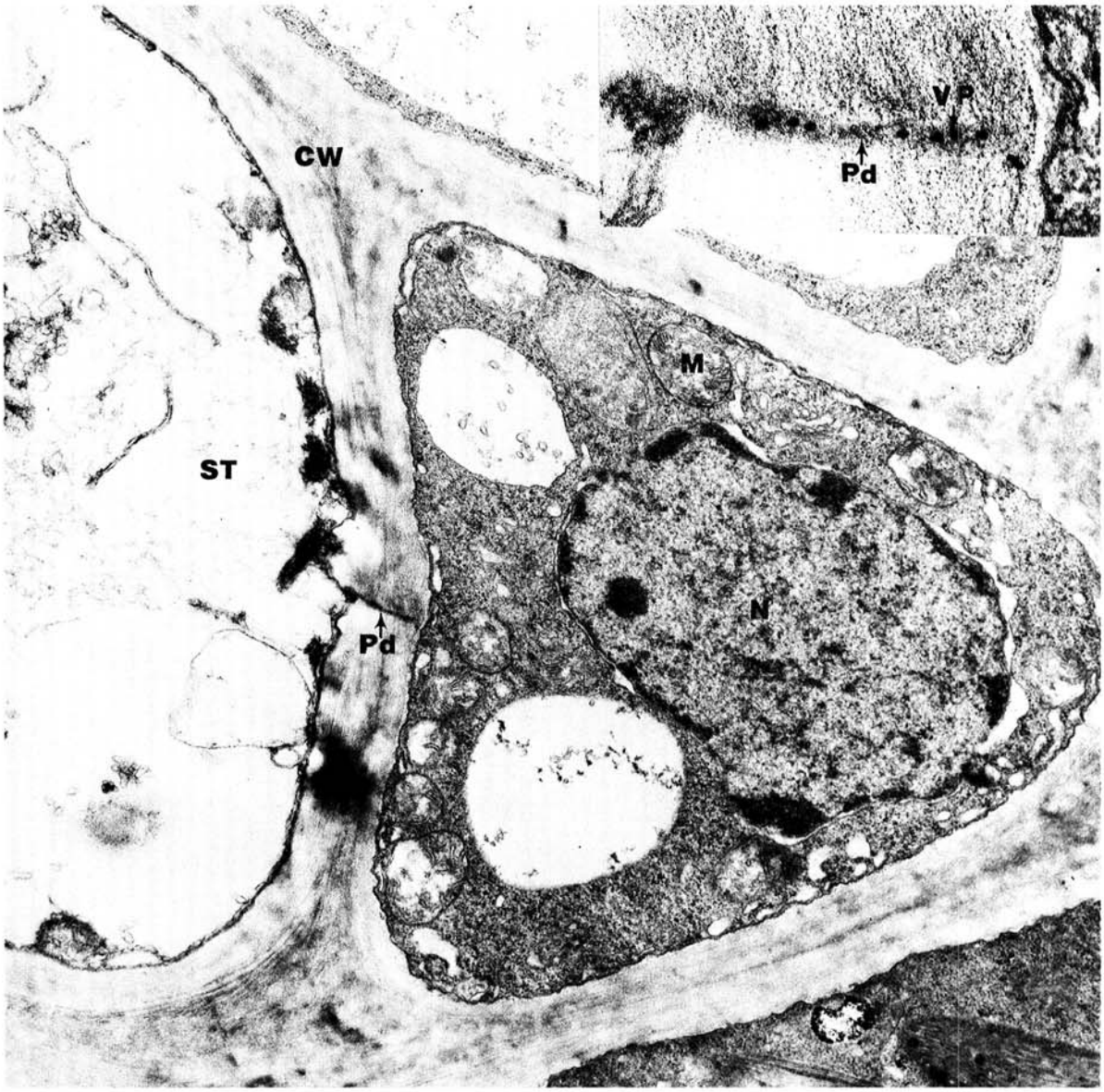


**Fig. 3.** Cross section through a phloem fiber in the stem of tobacco ringspot virus-infected soybean. Note virus particles passing through a plasmodesma connecting a sieve tube and companion cell (×18,000). The inset is an enlargement of the plasmodesma and virus particles (×90,000). ST = sieve tube; CW = cell wall; M = mitochondria; N = nucleus; Pd = plasmodesma; VP = virus particle.

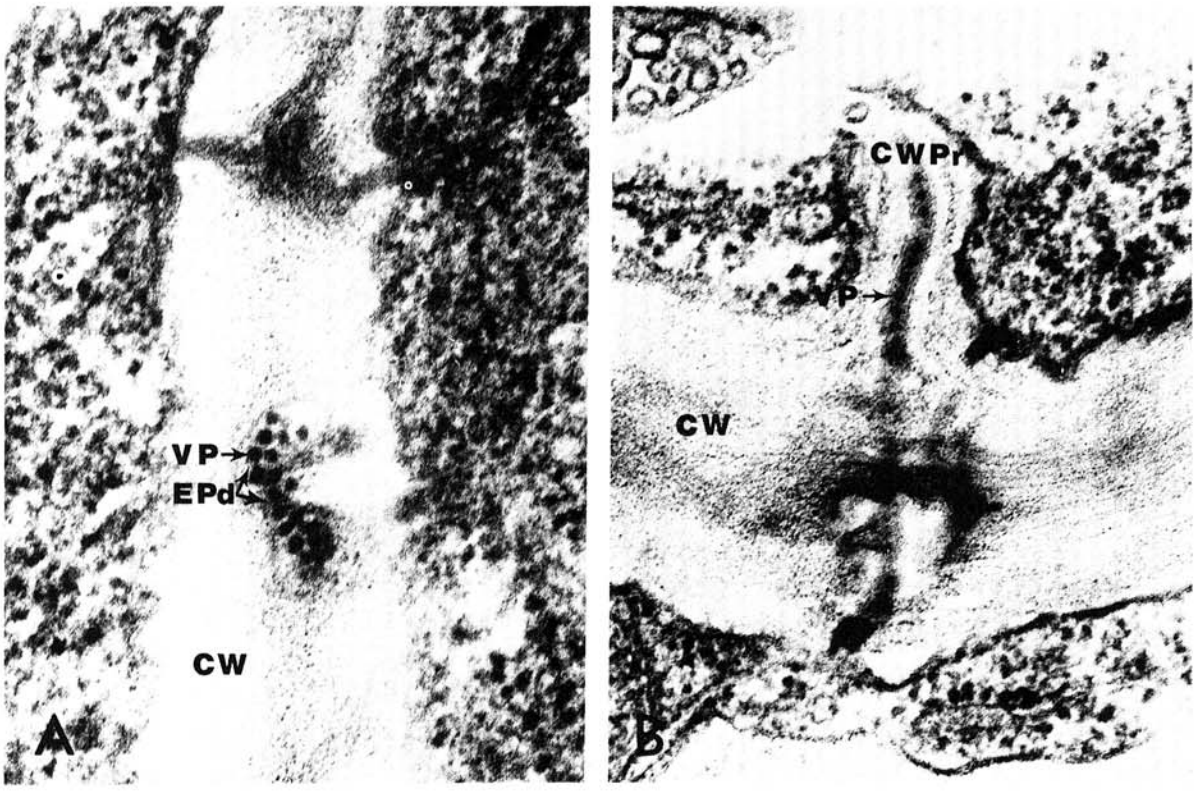


**Fig. 4.** Cross section of the stem of TRSV-infected soybean with virus particles. **A)** Enclosed in enlarged plasmodesmata between two companion cells (×90,000); and **B)** Enclosed in a tubule in a cell wall protuberance (×75,000). CW = cell wall; CWP = cell wall protuberance; EPd = enlarged plasmodesmata; VP = virus particles.

second nodes of soybean is probably the result of both anatomy and physiology of the soybean stem. Cell-to-cell spread of TRSV undoubtedly occurs in the stem of soybean, but the rate would be very slow and would not account for a significant amount of upward translocation of the virus. The anatomical studies indicate that when stems are inoculated with TRSV below the first node, the virus probably moves into the continuous traces that pass the cotyledonary node if rapid translocation to primary leaves or top leaves occurs. The other four traces enter the short-lived cotyledons and therefore are not continuous with the upper parts of the plant. Depending on which phloem bundles the virus enters in the continuous traces, it will either move into the primary leaves or pass directly to the upper portions of the plant. The presence of aggregates of virus particles in the vascular parenchyma cells, virus particles passing through the plasmodesmata connecting these cells, and virus aggregates and crystals in the sieve tubes of the phloem seem to confirm these assumptions.

Bidirectional flow of photosynthetic products may occur in phloem bundles (4, 6). Transport of TRSV in the phloem seems to be in the direction of movement of these products. Therefore, in stem infections it is possible for virus to enter phloem bundles in which the direction of flow is either up toward the apex of the stem or down toward the roots. As the plant ages, and more trifoliolate leaves develop, the direction of flow in the majority of phloem bundles at or below the second node is toward the roots. This assumption is supported by studies of translocation of photosynthetic products in soybean by Thaine et al. (22) and red kidney bean by Biddulph & Cory (4). In each case, they found that lower leaves exported the majority of their photosynthetic products to the roots, upper leaves exported to the stem apex, and intermediate leaves exported their products in both directions. Therefore, the chance of TRSV being translocated upward from below the first or second nodes, or even higher nodes in older plants, decreases as the plant grows older, even though more phloem bundles are available.

Although some plants become systemically infected within 1 week after stem inoculation below the first node more time is required in most plants. Still, there is a definite inoculation age limit for systemic invasion of the soybean plant. It is difficult to explain why there is an increase up to 6 weeks after inoculation in the number of systemically infected plants among those which were inoculated below the first node when they were less than 12 days old; whereas, plants inoculated at 12 days of age

or older do not show any appreciable upward translocation of the virus in 6 weeks. Perhaps this is related to decreased susceptibility of cells or tissues of the aging stem to virus invasion, or invaded cells produce a limited quantity of virus insufficient for distribution throughout the plant. Decreased susceptibility of other plant parts or tissues because of increased age has been reported for TRSV (18). If translocation of photosynthetic products occurs from a source to a sink according to an osmotic gradient (7), and if virus is translocated in the phloem as a part of the resulting mass flow, the amount of virus source from older infected cells may not be sufficient for translocation of virus to young leaves near the top of the plant and their infection to occur. Also, in younger plants the tissues of the lower stem should be more metabolically active, and may aid the transport of virus into sieve tubes with the translocation stream. In some instances virus seems to be translocated upward to young leaves emerging from intermediate nodes before being translocated to the top leaves.

Replication of TRSV in immature sieve tube cells in stem tissue and subsequent translocation upon maturity of these cells could also explain lack of systemic infection in plants inoculated at 12 days of age or older; i.e., it is possible that systemic infections occur only if the phloem parenchyma cells, companion cells, and/or sieve tubes become infected before they reach a certain age, even though parenchyma cells of the cortex and epidermis of the stems remain susceptible for a longer period.

Increased thickness of sclerid fibers, which may or may not be susceptible to virus invasion, could also slow cell-to-cell movement of virus from the cortex to phloem tissue. Once the companion cells and related parenchyma cells become infected, it may be some time before virus enters phloem sieve tubes in which the direction of flow is upward.

The increase in systemic infections when stems were inoculated by a combination of pricking and rubbing probably resulted from use of a more concentrated inoculum and inoculation of a larger surface area of the stem. The presence of more primary infection sites should improve the possibility that virus would enter phloem bundles continuous to the upper portions of the plant before the plant reached the age at which no upward translocation occurred from below the second node. A similar relation of virus inoculum concentration and number of primary infection sites to amount of systemic infection has been noted by Bennett (2) with sugar beet yellows virus and by Schneider (19) with TRSV in pinto bean.

TRSV particles arranged in single file in tubules which passed through the plasmodesmata of root tip cells of *Phaseolus vulgaris* was reported by Davison (8) as a means of cell-to-cell movement of TRSV. In this study of soybean stem tissue the presence of TRSV particles without associated tubules within the plasmodesmata, sometimes in clumps, indicates that tubules are not necessary for movement of TRSV through plasmodesmata.

The cell wall protuberances enclosing tubules containing TRSV particles are similar to the cell wall protuberances and cell wall thickenings with tubules containing bean pod mottle virus particles that Kim & Fulton (14) observed in bean leaf cells. The significance of these cell wall protuberances and tubules containing virus particles in soybean stems is not apparent, but such structures may occur commonly in plant tissues infected with small polyhedral viruses.

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