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THE RELATION OF VIRUSES TO PLANT TISSUES

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INTRODUCTION

The subject of the relations of viruses to plant tissues has been approached from several different angles, beginning with the early work of Allard (1915) who showed that the virus of tobacco mosaic invades nearly all of the plant parts.

More recent work has provided much additional information regarding the invasion of plants by the virus of tobacco mosaic and by numerous other viruses and has extended knowledge of the subject into the field of the relation of viruses to specific tissues of the plants involved. This information is scattered through a large volume of literature and much of it is incidental to the consideration of other phases of the diseases resulting from virus activity.

It is the purpose of this paper to assemble as much as possible of the evidence bearing on the relation of plant viruses to specific tissues and to point out some of the deductions that may seem justified by the information that has been made available up to the present time.

TISSUES INVADED BY VIRUSES

It is generally considered that plant viruses, with a few notable exceptions, are systemic and that when introduced into a susceptible plant they usually invade all of the plant parts. However, even with so-called systemic viruses there is evidence that the various types of tissues differ in the amounts of resistance offered to invasion. This resistance varies, depending on the plant and on the virus involved. Since, so far as known, viruses do not normally invade non-living tissues, a discussion of the invasion of plants by viruses may be limited to a consideration of the three major types of living tissues, namely, meristematic, parenchymatous, and conductive tissues.

Meristem. The determination of the presence or absence of virus in meristematic tissue offers certain difficulties that, although probably not insurmountable, have not yet been satisfactorily overcome. Maturation is so rapid at the growing points that only small quantities of meristem are available for direct determination of virus content. Isolation of relatively small groups of meristematic cells with no contamination from adjacent tissues requires patience and skill. However, it would seem that a direct determination of the presence or absence of virus is a possibility in the meristem of root tips and in certain types of callous formations.

There is evidence tending to indicate that after introduction into any part of the plant, viruses usually move rapidly to the growing points where symptoms appear first on the new growth. Other evidence indicates that sometimes young succulent parts are more susceptible to infection than older more mature parts. These considerations have led some investigators to assume that rapid multiplication of viruses is associated with relatively large quantities of meristem. Caldwell (1931) states that it is generally conceded that presence of meristematic tissues is necessary for active multiplication of the virus or tobacco mosaic and suggests that slight tendency to faster upward movement found with this virus may be associated with the increased rate of multiplication of the causal agent in meristematic tissue. However, it is well known that this virus is able to multiply in mature leaves and stems.

Matz (1934) found that the juice from the rolled inner white and brittle portions of leaf bases in the lower regions of the pseudostem of sugarcane plants affected by sugarcane mosaic, apparently had a lower concentration of virus than juice from mature green leaves, midribs, leaf sheaths, and true stems. Also (Matz 1935) the white, brittle portions of the inner leaf bases immediately above the apex of the stem gave evidence of being less receptive to infection in artificial inoculation tests than surrounding tissues. Juice from green parts of diseased plants mixed with juice from immature parts of the pseudostem of healthy plants was less infectious than mixtures of juices from the green parts of diseased and healthy plants. This evidence indicates that meristematic tissue of sugarcane is unfavorable to the virus of mosaic.

Grainger (1934) emphasized the lack of direct evidence that would indicate that viruses are able to enter meristematic tissue.

More recently, however, Lackey (1938) reported finding relatively high concentrations of the virus of curly-top in the root tips of beets and beans beyond the region of differentiating phloem. If this virus occurs in meristem of root tips in the relative concentrations suggested, it is evident that the meristem of the root tips of beet and bean plants has a considerable degree of permeability as well as great resistance to injury. The close association of the virus with the phloem in the more mature parts of the plant indicates that the virus is inactivated in most of the tissues derived from infected meristem.

Sheffield (1933) found that in certain Solanaceous plants affected by aucuba mosaic, the meristematic tissue of diseased plants appeared to be like that of healthy plants. Incipient inclusion bodies were not found until plastid development was well advanced, but the virus apparently entered some of the cells early enough to inhibit development or cause destruction of plastid primordia, thus giving rise to the chlorotic leaf areas. In the green areas the plastids were normal, indicating that the virus entered the cells at a later stage of development. Under such conditions it must be assumed that the more actively dividing meristematic cells were free of virus.

Valleau (1935) states that the meristematic tissue of the growing point of tobacco plants appears to be nearly immune to the virus of tobacco mosaic, and suggests also that failure of this virus to enter the seeds may be the result of inability of the virus to invade meristematic tissue.

Apparently there is no evidence of a cytological nature that indicates the presence of any virus in cells of the meristematic regions of affected plants. The continued normal functioning of the meristem of the growing points and cambium regions of most virus-affected plants, indicates that if viruses are present in the meristem they rarely cause appreciable direct injury to this type of tissue.

The more rapid movement of viruses from points of introduction to the growing points of plants that is found frequently may be associated with the transport of elaborated foods to these regions. This possibility is discussed further in another section of this paper.

Parenchyma.[1] Various types of parenchymatous tissues are un-

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[1] In this paper the epidermis, although not usually classified as parenchyma, is treated as if it were a type of parenchymatous tissue. This seems justified in the interest of brevity and simplicity, since the epidermis partakes of most of the characteristics of parenchyma and since its relation to viruses is similar to that of true parenchyma.

doubtedly extensively invaded by certain plant viruses. The rubbing of leaves of tobacco plants with a cloth saturated with juice from plants affected with common mosaic, results in infection. This method of inoculation introduces the virus into trichomes and other epidermal cells. The virus passes from the injured cells into cells of the palisade and mesophyll and finally enters the vascular elements through which it is transported rapidly to other parts of the plant. It seems certain that the virus multiplies in parenchymatous tissue and it seems reasonable to expect that all viruses that are capable of producing infection through injured epidermal cells are able to move and multiply in the ground tissue of the plant. It may be suspected further that all viruses that cause mottling or local lesions in leaves are able to invade parenchyma, even though they may not be readily transmissible by mechanical inoculation.

However, there are diseases, leaf-curl of raspberry, peach-yellows, sugar beet curly-top, aster-yellows, and others, caused by virus, that do not produce local lesions or mottling and with which infection through injured epidermal cells apparently does not occur. Where these viruses remain active in plant extracts, the rubbing method of inoculation mentioned in connection with tobacco mosaic undoubtedly serves to introduce them in an active state into epidermal cells. The obvious conclusion is that where such viruses remain active in expressed plant juice but fail to produce infection when introduced into parenchyma cells, they are probably unable to multiply in parenchyma tissue or to migrate through parenchyma from the cells into which they are introduced.

Other evidence supports the view that where infection by mechanical means is difficult, often little or no virus occurs in the parenchyma. *Eutettix tenellus* (Baker), the vector of the virus of curly-top of sugar beet, rarely obtained virus when its feeding was restricted to parenchyma of diseased plants (Bennett, 1934) or when its feeding was restricted to extracts of juice from parenchyma of diseased plants (Bennett and Esau, 1936). Also, the virus failed to pass through the woody cylinder of tobacco stems in periods some of which were longer than one year. Although this evidence does not prove complete absence of the curly-top virus from all parenchyma, it indicates that, at most, not more than relatively low concentrations of virus occur in the types of parenchyma tested. It seems reasonable to suspect that a number of viruses of this general type may have similar tissue relationships.

Vascular tissue. The vascular bundles of plants serve to rapidly transport viruses to various parts of the plant, and in some instances they appear to be the sole channels for virus transport. Due to differences in anatomical structure and physiological functioning of the xylem and phloem of vascular bundles it would be expected that these two parts of the conductive strands would bear very different relations to viruses.

Certain investigators have suggested that the xylem may be the path of dispersion of viruses through the plant, but little direct evidence supporting this view has been presented. Johnson and Mulvania (1924) attempted to force virus of tobacco mosaic from the xylem of tomato plants through the hydathodes of the leaf by placing the root system under a hydrostatic pressure of 200 pounds per square inch. Liquids obtained from the hydathodes by this method proved to be infectious but contained less virus than extracted plant juice. It seems probable, as Johnson and Mulvania point out, that the liquid from the hydathodes may have been contaminated by content of living cells injured by the high pressures employed.

Later work by Caldwell (1931) has shown that water naturally guttated from tomato leaves affected by aucuba mosaic contained no virus, whereas liquid guttated under pressure contained virus. Grainger (1933) obtained similar results using the virus of tobacco mosaic in tomato.

Other evidence indicates strongly that viruses are not normally found in the tracheae of the xylem, but there is some indication that they may occur in the xylem parenchyma. Numerous attempts have been made to infect plants by filling the tracheae with liquids in which viruses were suspended. However, infection has not resulted from this method of introducing viruses into plants when the plants were not injured after the viruses were introduced. Caldwell (1930, 1931) induced liquids containing the virus of aucuba mosaic to enter tomato plants through the cut ends of petioles and pass to various parts of the plant through the xylem. No symptoms of disease appeared when the plants were not injured further. However, infection was produced readily when the xylem was crushed and the tracheal content allowed to escape into adjacent tissues. When this virus was inoculated into plants by the usual method of rubbing, it did not pass a part of the stem that had been killed by steaming. When placed in the xylem, however, the virus passed the steamed areas and caused symptoms on the other side of the steamed areas when released from the tracheae through injuries. Similar results were obtained with steamed stems of tobacco using the virus of tobacco mosaic.

This evidence shows that these viruses were unable to pass out of unbroken tracheae and enter adjacent cells. Conversely, it does not seem probable that they would be able to pass from living cells into tracheae. This indicates that viruses do not occur normally in that part of the xylem chiefly concerned with the movement of water and mineral elements. However, Matsumoto and Somazawa (1933) presented evidence indicating that the virus of common mosaic of tobacco occurs in the woody cylinder of tobacco plants. In this case the virus was present probably in the wood parenchyma and in the medullary rays. Bennett (in press) found that the virus of tobacco mosaic is able to pass either longitudinally or radially through the woody cylinder of stems of Turkish tobacco. This was true also of the virus of cucumber mosaic in *Nicotiana glauca*. Perhaps viruses that occur generally distributed in parenchyma would be expected to invade parenchyma of the xylem regions.

Evidence of a close relationship between viruses and the phloem portion of the vascular bundle is very strong. The majority of insects that are vectors of plant viruses seek out and feed on the phloem. This is strikingly evident in the case of vectors that transmit viruses not easily transmissible by mechanical means. Certain viruses, such as the virus of spotted-wilt transmitted by *Frankliniella insularis* (Bald and Samuel, 1931) and the virus of pineapple yellow-spot transmitted by *Thrips tabaci* (Linford, 1932), are transmitted by insects that presumably feed on parenchyma, but these viruses are also transmissible by mechanical means, and introduction of virus into parenchyma is probably sufficient for infection.

Most insects that feed on the phloem are admirably equipped by nature not only to remove large quantities of material from the phloem but also to introduce appreciable quantities of their own secretions into the phloem as well as into cells of surrounding tissues. The marked effectiveness of phloem-feeding insects as vectors and the complete dependence of certain viruses on this type of vector for dissemination point strongly to an intimate relationship between the phloem and the viruses that are transmitted.

Holmes (1932) showed by the starch-pattern method of following virus movement that the virus of tobacco mosaic bears a very decided relation to the veins of the leaf in some of the earlier stages of its invasion of the plant. Samuel (1934) found a similar condition in tomato plants affected with tobacco mosaic. The rate of movement following contact of the virus with the veins and the subsequent path of movement clearly indicate that the faster rates of movement occur in the veins. In view of the evidence indicating absence of virus in the tracheal elements of the xylem, it seems evident that the rapid movements of these viruses through leaves and stems occur in the phloem.

Ringing experiments show (Bennett, 1927) that the leaf-curl virus of raspberry is unable to move through the woody cylinder of raspberry canes. Somewhat similar experiments indicate (Bennett, 1934) that the virus of curly-top is unable to move longitudinally or laterally through the woody cylinder of *Nicotiana glauca* or *N. tabacum* but that it passes readily from internal to external phloem, or *vice versa,* through the union of the two types of phloem in the leaf traces. As already pointed out, the curly-top virus occurs in very low concentrations, if at all, in the cells of the parenchyma of the petiole, pith of the crown, and flowering stalk of beet. Exudate produced naturally on diseased petioles and probably derived originally from the phloem and exudate derived from the severed ends of vascular bundles of diseased beet roots has a very high virus content.

With most viruses the evidence points to the phloem as the tissue through which rapid invasion takes place and in some plants the tissue in which virus occurs in greatest concentration. The phloem is apparently well adapted to the rapid distribution of virus to all parts of the plant when conditions are favorable for movement.

CLASSIFICATION OF RELATIONS

Approaching the subject of tissue relations of viruses from a somewhat different viewpoint and considering only phloem and parenchyma, viruses seem to exhibit three main relationships to plant tissues. These relations may be classified as follows: (1) a relation in which virus is more or less restricted to parenchyma, (2) a relation in which virus is more or less restricted to the phloem, and (3) a relation in which virus occurs extensively distributed in both phloem and parenchyma.

Restriction to parenchyma. A virus restricted to parenchyma in all of its host plants would be greatly handicapped. Movement through the parenchyma is known to be relatively slow, and the time required to invade all of the parts of a plant by travel through the parenchyma probably would be quite long. Thus, the amount of inoculum would be more limited, and spread from plant to plant would occur less often than if the virus were able to invade the plant through the phloem. If a virus of this type were evolved it would require special conditions to enable it to survive, especially if not seed-transmitted and if its host plants were annuals.

The virus of tobacco necrosis, as described by Smith and Bald (1935) and by Smith (1937), shows some extremely interesting relations to infected plants and may prove to be closely limited to parenchyma. This virus is able to persist for long periods in certain greenhouse soils and is resistant to the ordinary agents in the soil. It attacks tobacco and a number of other plants but is usually restricted to the roots, although in young plants it may move into the stems and cause necrosis of stem and leaf tissues, sometimes resulting in death of the plant. When inoculated into leaves it causes necrotic lesions in a wide range of plants but produces systemic infection only in French bean (*Phaseolus vulgaris*). Since natural infection usually takes place from the soil and since the virus shows a high degree of restriction to roots, it seems probable that it is largely confined to parenchyma and unable to pass into the phloem and move rapidly into the tops of affected plants.

There is evidence that there are viruses capable of extensive tissue invasion in some of their host plants but restricted to parenchyma in other plants. Holmes (1929) showed that the virus of tobacco mosaic was usually localized in the inoculated leaf of *Nicotiana glutinosa,* and Caldwell (1932) found that the virus of aucuba mosaic produced local lesions in this species but was unable to produce systemic infection. Several other cases are known in which viruses produce local lesions on certain host plants but fail to invade the plant systemically.

There are viruses also that are able apparently to produce systemic infection under certain conditions but restricted to parenchyma under other conditions. Smith (1932) found when the virus of spotted-wilt of tomato was inoculated into petunia leaves by rubbing or by means of the insect vector, *Thrips tabaci,* local lesions resulted, but usually systemic infection did not occur. On the other hand, when the virus was introduced into the stems by needle punctures systemic infection resulted but no local lesions were produced. This seems to constitute a case in which the virus is capable of remaining active in either phloem or parenchyma but passes from one type of tissue to the other with difficulty. Rubbing the leaves with a virus suspension or inoculating them using thrips would ordinarily introduce the virus into the more superficial parenchyma cells. In these cells it apparently multiplied and caused necrotic areas, but since systemic infection was not produced it probably did not enter the phloem. Introduction of the virus into the stem through needle punctures might, in some instances at least, place the virus in the phloem. From the point of introduction it would be carried to other parts of the plant and become systemic. From such systemic infections, however, local necrotic spots did not develop, which suggests that although the virus was able to invade the phloem network of the plant it was not able, at least in the leaves mature at the time of inoculation, to pass out of the phloem in quantities sufficient to produce local lesions.

Also in recent work it has been found that a strain of cucumber mosaic virus when introduced into mature leaves of sugar beet by rubbing produces only local lesions. The local lesion phase of the disease can be perpetuated indefinitely. When the virus is introduced into the plants by aphids, however, a systemic infection frequently results that causes a severe type of mottling but produces no local lesions of the type resulting from inoculation by the rubbing method.

The chance of survival of a virus limited to parenchyma in all of its host plants would seem to be greater in perennial plants, especially in vegetatively propagated perennials. One virus which apparently has this limitation occurs in peach. Hutchins (1939) found that when whole root sections from peach trees having phony peach were grafted onto roots of healthy trees the disease was transmitted in all cases where union took place; but, when bark from diseased roots was grafted onto healthy roots no transmission occurred. This indicates that the virus of phony peach is restricted to the woody cylinder. Since it is reasonable to conclude that the virus is closely confined to living tissues, it seems probable that it moves and multiplies in the wood parenchyma, or in the medullary rays or both.

The tissue relationships of this virus are all the more interesting because of the fact that it is apparently not present in the tops of affected plants (Hutchins, 1929). Why the virus should occur in the woody cylinder of the roots and not in the woody cylinder of the limbs is an interesting question. Kunkel (1935) suggested that the virus may be inactivated in the parts above ground during periods of high summer temperatures. Hutchins and Rue (1939) presented evidence indicating that the virus is destroyed by subjecting dormant infected trees to a temperature of 48° C. for a period of 40 minutes. The effect of lower temperatures over longer periods has not been determined. It may be pointed out, however, that since the virus is presumably limited to parenchyma, spread probably is slow, and several months might be required for it to move from the roots to the tops of an average size tree, even under conditions favorable for maximum rates of movement. Therefore, summer temperatures high enough to cause inactivation of the virus in the above ground parts would be expected to cause restriction of the virus to the roots and lower parts of the trunk.

Restriction to phloem. There are probably several viruses that are more or less closely limited to the phloem in their increase and movement in the plant. Of those that probably have this limitation, the virus of raspberry leaf-curl and the virus of sugar beet curly-top have been studied most extensively in this connection. Studies (Bennett, 1927, 1934) have shown that these viruses may be confined to certain parts of an infected plant by destroying the phloem connections between the inoculated portion and other parts of the plant at the time of inoculation.

The virus of curly-top passes readily through either the internal or the external phloem of the stem of both *Nicotiana tabacum* and *N. glauca,* but it is unable apparently to pass from one type of phloem to the other through the medullary rays or other tissues normally found in the woody cylinder of the internodes of the stem, although it makes this transition without any measurable delay by means of the union of the external and internal phloem in the leaf traces. In both beet and tobacco the virus of curly-top occurs in relatively high concentrations in the phloem and is absent from or present in only low concentrations in the parenchyma adjacent to vascular bundles of the petiole, crown, and flower stalk. In beets affected by curly-top, Esau (1933) observed necrosis only in the primary and secondary phloem and pericycle. She interpreted changes occurring outside the phloem as secondary responses to necrotic conditions in the phloem and concluded (1933, 1935, 1935*a*), on the basis of extensive anatomical evidence, that the virus is active mainly in the phloem.

It seems probable that there are other diseases besides leaf-curl of raspberry and curly-top of sugar beet that are caused by viruses closely limited to the phloem. This conclusion is reached on the basis of the characteristics that diseases caused by phloem-limited viruses would be expected to manifest.

In general, symptoms should be those characteristic of diseases arising from disturbances in the phloem. Typically they might include phloem necrosis, vein distortion, leaf rolling, curling and crinkling due to growth disturbances in the veins, and yellowing and dwarfing of parts and of entire plants, but probably no mottling of the mosaic type would be expected. Viruses of this type would not be seed-borne, for, since they do not occur in tissues outside the phloem, they would not be able to enter the gametes or to pass through the meristematic or parenchymatous bridge separating the mother plant from the young sporophyte. It is significant in this connection that no virus which does not produce local lesions or mottling has been shown to be transmitted through seeds.

Transmission by placing virus in the superficial cells of the leaf, as in the rubbing method of inoculation, would not result in infection, and inoculation by means of needle punctures would rarely produce infection since it seems to be difficult to introduce virus directly into the phloem. It is recognized, however, that certain viruses that probably are not limited to the phloem also fail to produce infection when introduced into epidermal cells, perhaps because of rapid inactivation when removed from the living cell or inability to reproduce and move in the presence of products of the injured cells into which they are introduced. For this reason, failure of mechanical inoculation to produce infection, although characteristic of phloem-limited viruses, can not be accepted as conclusive evidence that a specific virus is limited to the phloem.

Viruses that are limited to the phloem should be almost exclusively insect transmitted, and vectors should be relatively few in number and exhibit a greater degree of specificity in virus transmission than is found in vectors of viruses not limited to the phloem.

It seems probable that only insects that habitually feed on the phloem and that permit virus to pass through their bodies and to be injected into the plant through the medium of their saliva, would qualify as effective vectors.

With these points in mind, it seems probable that such diseases as peach-yellows, little-peach, peach-rosette, potato leaf-roll, yellow-dwarf of potato, aster-yellows, cranberry false-blossom, peanut-rosette, and spike-disease of sandal are caused by viruses that may be more or less closely limited to the phloem. Indirect evidence, recently published, supports this view in respect to peach-yellows, little-peach, peach-rosette, and potato leaf-roll.

Kunkel (1938), in the transmission of virus diseases of peach by budding, found that the contact period between bud and twig required for mosaic transmission was usually 2 to 3 days, whereas the contact period required for the transmission of yellows, little-peach, and rosette was 8 to 14 days. Since the virus of mosaic would be expected to occur in the parenchyma, it would pass out of the infected buds into healthy tissue as soon as parenchymatous bridges were available, whereas the viruses of the other three diseases, if restricted to the phloem, would pass from infected buds into healthy tissue only after phloem bridges were available, which would be sometime after parenchymatous unions had been formed.

In the transmission of potato viruses by vectors, Dykstra and Whitaker (1938) found that four species of aphids, *Myzus persicae, M. solani, M. circumflexus,* and *Macrosiphum* (*Illinoia*) *solanifolii,* under certain conditions are effective vectors of certain mosaic viruses of potato. The first three were also effective vectors of the potato leaf-roll virus but the fourth generally failed to transmit the leaf-roll virus though occasionally a fairly high percentage of infection was obtained. The first three species named above were found to feed on the phloem but the fourth species fed on the phloem in less than 50 per cent of the cases noted. A feeding relation of this latter type would be expected to result in reduced efficiency in the transmission of a phloem-limited virus.

The yellow-dwarf virus of potato, transmitted by the leafhopper *Aceratagallia sanguinolenta,* may furnish an example of a virus closely limited to phloem only in certain hosts. Black (1938) showed that on potato and crimson clover the virus is transmissible with extreme difficulty, if at all, by the ordinary methods of inoculation that serve only to introduce viruses into epidermal cells; whereas these methods were very effective in transmission of the virus to a certain variety of *Nicotiana rustica.*

It was concluded that probably the main requirement for infection of potato and crimson clover is introduction of the virus into the phloem, and that for infection of *N. rustica* introduction of the virus into the phloem is not necessary.

Symptoms of the disease on potato and crimson clover are typical of those caused by phloem-limited viruses and consist of yellowing and dwarfing in potato and of vein clearing, yellowing, and dwarfing in crimson clover. Symptoms on *N. rustica* are more typical of those caused by parenchyma invasions and consist of yellow spotting and necrotic lesions. Systemic infection indicates the virus also occurs in the phloem. The relatively high concentration of virus found in juice from infected plants of *N. rustica* as compared with concentrations found in juice from potato and crimson clover is further indication that the virus is more extensively distributed through living tissues in *N. rustica* than in potato and crimson clover.

Occurrence in both parenchyma and phloem. Perhaps common tobacco mosaic is the best known example of a virus disease in which the causal agent occurs in both parenchyma and phloem, but there are numerous other viruses that have a similar tissue relationship. The virus of tobacco mosaic produces infection through trichomes or other epidermal cells and passes from the epidermis through other types of parenchymatous tissue before it reaches the phloem in which it evidently travels at its most rapid rate.

Holmes (1932) has shown that the rate of initial spread following introduction into epidermal cells is slow and roughly equal in all directions until the larger veins are reached. After the virus enters a vein it moves away from the original lesion very rapidly. A limited period of delay in entering small veins and a close association with the veins in the earlier stages of systemic invasion suggest the presence of cells around the phloem that are permeable to the virus with a certain amount of difficulty. The permeability of these cells apparently varies somewhat at different points, since Holmes has shown that as the virus passes away from the place of entrance it is at first closely restricted to the veins for the most part but escapes at intervals to widen considerably the path of invasion in certain parts of the leaf. Samuel (1934) found a similar unequal spread of virus from the veins into the neighboring parenchyma of tomato leaves.

Probably all of the viruses that cause mosaic mottling first extensively invade plants through the phloem and later spread into other tissues where they continue to increase and often reach relatively high concentrations.

Viruses of this type might be expected to produce symptoms that would be evident in either parenchyma or phloem or both. However, the most evident symptoms in most instances seem to be those resulting from disturbances in the parenchyma and consist as a rule of local lesions of various types, and of mottling. Phloem necrosis and other phloem disturbances occur more rarely.

The inconspicuous character or absence of symptoms in the phloem of plants infected by viruses that cause mottling or local lesions suggests that some of these viruses may occur only in low concentrations in the phloem. Perhaps a virus that is equipped to multiply rapidly in an acid medium such as parenchyma would not multiply equally well in an alkaline medium such as the phloem. If the phloem content were decidedly unfavorable, movement through the phloem might occur only under special conditions. This may account for some of the cases of delayed systemic infection and partial invasion of plants by certain viruses.

Storey (1938) showed that *Cicadulina mbila,* the vector of the virus of streak-disease of maize, normally feeds on the phloem and must feed on the phloem in order to transmit. When it was allowed to feed on chlorotic areas of infected maize leaves it picked up virus but when its feeding was restricted to the normal green areas of the leaf it was unable to acquire virus from such areas. This was true regardless of whether feeding was confined to parenchyma or permitted to extend into the phloem. It seems quite improbable that the virus could be so localized that its occurrence in the phloem would be restricted to areas surrounded by infected parenchyma cells. It seems probable, therefore, that the insect acquires virus from infected parenchyma cells and that it cannot pick up enough virus from the phloem to enable it to become a vector, although the virus undoubtedly occurs in the phloem and moves in this tissue very rapidly. This evidence suggests relatively high concentrations of virus in the parenchyma of chlorotic spots, low concentrations or absence of virus in normal green parenchyma, and very low concentrations of virus in the phloem.

Further information regarding the relative concentration of virus in phloem and parenchyma of plants affected by other viruses would be of interest. In certain plants this information could be obtained by comparing concentrations of virus in expressed sap with those in phloem exudate.

Viruses that occur in both phloem and parenchyma should be readily inoculable by mechanical means except with those that are easily inactivated by products of injured cells or by other factors encountered in the transfer of viruses from one plant to another.

Insects that feed chiefly on the phloem and that are able to pass viruses through their bodies and reintroduce them into the plant through the medium of their saliva would be effective in transmission of viruses that occur in both phloem and parenchyma. However, passage through the insect may not be so important as with phloem-limited viruses.

Doolittle and Walker (1928) showed that *Aphis gossypii* loses the virus of cucumber mosaic in the first or second short-interval transfer on healthy plants, and Bennett (1932) found a similar condition with *Amphorophora rubi* in its relation to the virus of mosaic of raspberry. Although it is possible that these results are due to the inability of the viruses involved to remain active in association with the vectors, the possibility remains that the viruses may have been carried only on the mouthparts and were washed off when the insects fed on healthy tissue. This latter interpretation is supported to a certain extent by results obtained by Fukushi (1939) who found that *Aphis laburni* loses the virus of red clover mosaic in feeding periods of 10 to 30 minutes on healthy plants but retains it for about an hour when it does not have access to food plants.

Mechanical transmission by contaminated mouthparts was suggested by Severin (1931) to explain rare cases of acquisition and transmission of the curly top virus by *Eutettix tenellus* in periods that may have been too short to permit the virus to pass through the insect.

If certain insects are able to transmit viruses by mechanical transfer on their mouthparts, they would probably be more effective in transmission of viruses that occur in parenchyma than of viruses restricted to phloem.

Where virus occurs in both phloem and parenchyma, it may be acquired from parenchyma, and infection may be produced by properly introducing it into parenchyma. Therefore, certain types of insects such as thrips, certain species of aphids and possibly other insects that feed chiefly or exclusively on parenchyma, are able to act as vectors. Even phloem-feeding insects may be able to function as vectors without actually feeding on phloem, as shown by Watson (1936) who found that *Myzus persicae* is able to acquire the Hy. III virus by feeding only on parenchyma of diseased plants and to introduce it into healthy plants in feeding intervals too short to permit the mouthparts of the insects to reach the phloem. In view of the possibility that phloem may be less favorable than parenchyma to multiplication of this type of virus, it would be of interest to have more information regarding the effectiveness of insect introduction of virus into parenchyma as compared with introduction into phloem.

Much evidence points to the conclusion that the vector relations of viruses that occur in both phloem and parenchyma are much less specific and limited than those of phloem-limited viruses. Drake, *et al.* (1933) showed that more than 50 species of aphids transmit the virus of yellow-dwarf of onion, and many other viruses of the mosaic type are known to be transmitted by more than one species of vector. Kenneth Smith (1937*a*) lists 21 viruses transmitted by *Myzus persicae,* 10 by *Macrosiphum gei,* and 8 by *M. pisi.* With few exceptions, the viruses transmitted by these insects produce mottling or local lesions. The probability of infection following introduction of virus into either phloem or parenchyma may be an important consideration in the production of these results.

RATE OF MOVEMENT

The rate of invasion of tissues by viruses following their introduction into the plant is influenced by a number of factors, the most important perhaps being: (1) the kind of plant in which movement occurs, (2) the kind of tissue in which movement takes place, and (3) the virus involved. Since the significance of these factors may vary in different plants and since other factors may exert an influence, a wide variation in the rate of movement among viruses may be expected. This expectation is abundantly realized in the results of a considerable number of measurements of virus movement that have been made. As shown in table 1, these rates range from .18 cm. per hour, for one of the mosaic viruses of tomato, to 152 cm. per hour for the virus of curly-top of sugar beet.

The accuracy with which movement may be measured varies with different viruses. Accurate measurement of the rates of movement of mosaic viruses through the phloem is difficult because it is usually necessary to introduce these viruses into superficial cells, and entrance into the phloem is effected only after a period of relatively slow and variable movement through parenchyma. However, in tobacco the common mosaic virus is able to move as fast as 36 inches in 72 hours or at a rate of one-half inch per hour. The time interval includes the period of slow movement through leaf palisade and mesophyll and the rate of movement in the phloem is probably somewhat higher than that indicated. In tomato Kunkel (1939) estimates that this virus may move as fast as 7 inches per hour.

The movement of some of the leafhopper-transmitted viruses

TABLE I

RATES OF MOVEMENT OF VIRUSES IN PLANTS

Disease caused by virus	Plant in which movement occurred	Distance traveled in indicated time	Rate of movement; centimeters per hour	Reference
Mosaic	Tomato	8–18 inches in 10 to 15 days	1 to 2*	McCubbin and Smith (1927)
Mosaic	Raspberry	49 inches in 10 days	.52	Bennett (1932)
Curly Top	Sugar Beet	7 inches in 30 minutes	38.1	Severin (1924)
Curly Top	Sugar Beet	6 inches in 6 minutes	152.4	Bennett (1934)
Curly Top	Tobacco	24 inches in 48 hours	1.27	Bennett (1934)
Mosaic	Tobacco	13 cm. in 2 days	.29	Böning (1928)
Mosaic	Tomato	9 cm. in 2 days	.18	Böning (1928)
Mosaic	Tomato	14 inches in 2 hours	17.8	Kunkel (1939)
Streak	Maize	40 cm. in 2 hours	20.0	Storey (1928)

* Estimates given by McCubbin and Smith.

through the phloem can be measured with considerable accuracy, since the vectors are able to introduce the viruses directly into the vascular tissue. The viruses that have been found to move most rapidly are those of streak-disease of maize and curly-top of sugar beet. The virus of streak actually moved a distance of 40 cm. in 2 hours in a maize leaf and the virus of curly-top moved 6 inches in 6 minutes in a beet leaf. These movements are considered to have taken place in the phloem.

Some indication of the influence of the plant on rate of movement may be gained by comparing the movement of the virus of curly-top in sugar beet and in Turkish tobacco. In beet the virus moved 6 inches in 6 minutes or at a rate of 60 inches per hour and in tobacco it moved 24 inches in 24 to 48 hours or at a rate of not more than one inch per hour. Thus the virus moved at least sixty times faster in beet than in tobacco.

PATH OF VIRUS MOVEMENT

Virus activities such as multiplication and spread are believed closely linked with the chemical and physical processes that govern the functioning of living protoplasm. Within parenchyma cells, viruses appear to be closely associated with the cytoplasm. Livingston and Duggar (1934) concluded that the virus of tobacco mosaic probably is much more highly concentrated in the cytoplasm than in the vacuole. More recently Martin and McKinney (1938) found comparatively little virus in vacuolar sap and concluded that the cytoplasm contains most of the virus present in living tissue. This evidence, although not conclusive, indicates that cytoplasm is the chief path of movement of viruses in parenchyma cells.

Up to the present time, no evidence has been presented showing that any virus is capable of passing directly through the cellulose structure of the cell wall itself. Apparently infection does not take place through uninjured root hairs, trichomes, or other epidermal cells. The apparent inability of viruses to pass out of or into tracheae has already been mentioned. However, walls of tracheal and epidermal cells are somewhat specialized and perhaps would be expected to offer more resistance to virus passage than walls of certain other plant cells.

A certain amount of evidence is available on the resistance offered to virus passage by walls of cells within the leaf structure. By submerging parts of detached leaves of *Nicotiana glutinosa* in liquids containing virus of aucuba mosaic, and then reducing the pressure of the surrounding medium, Caldwell (1932) succeeded in introducing virus into intercellular spaces of the mesophyll. Local lesions characteristic of the disease developed on leaves treated in this manner in only a few instances and these lesions were attributed to accidental infection through injuries. The treated leaves were shown to be susceptible to infection when the virus was introduced through wounds. It was concluded, therefore, that the virus was unable to enter the cells from intercellular spaces by passage through the unbroken cell walls.

In other work bearing on this subject, Duggar and Johnson (1933) reported infection with tobacco mosaic virus through stomata of tobacco leaves. It was suggested that when virus suspensions were sprayed on leaves virus particles passed through the stomatal openings and entered the protoplasts from the substomatal cavities. The question was raised, however, as to whether the virus might not pass from the intercellular spaces into the cells through protoplasmic fibrils. Sheffield (1936*a*) was unable to verify these results and suggested that the infection obtained did not take place through the stomata but resulted from accidental infection through wounds made in the process of inoculation.

If viruses are unable to pass through the cellulose structure of all types of cell walls, the path of movement from cell to cell is limited to protoplasmic connections between cells. Plasmodesmata have been suggested by a number of investigators (Quanjer, 1931; Livingston, 1933; Drake *et al.*, 1934; Sheffield, 1936; and Martin and McKinney, 1938) as avenues of virus passage from one cell to another, and much evidence seems to support this concept.

If plasmodesmata are the sole avenues of virus travel through cell walls, as the evidence indicates, the implications are rather interesting. In view of the large number of plants in which it is known definitely that viruses invade at least most of the living cells of the ground tissue of the plant, it naturally follows that a general idea of the prevalence and distribution of plasmodesmata in plants can be obtained by observing the way in which virus invasions take place. This evidence supports the view of universal occurrence of plasmodesmata in flowering plants, and suggests also that plasmodesmata are well distributed throughout living tissues and serve as effective avenues for passage of materials from cell to cell.

The phloem as a path of virus movement was first suggested by Beijerinck (1898). Others (see Henderson Smith, 1930) have since presented evidence supporting this view. More recent work on rates of movement and on the restriction of virus passage by rings breaking phloem continuity, leaves little doubt as to the importance of phloem as an avenue of virus travel.

If the path of virus movement is the cytoplasm in the ground tissue of the plant, it might appear to follow that cytoplasm functions also in the movement of virus in the phloem. This may be true to a limited extent. It seems reasonable to expect that viruses that move in cytoplasm of cells of epidermis, palisade, and mesophyll, for example, would move in the same medium in other cells of these general types, and, therefore, would move in the cytoplasm of the phloem parenchyma.

However, there is no reason to suspect that viruses would move more rapidly in the parenchyma of the phloem than in other types of parenchyma. Rates of movement through the agencies available in parenchyma are not sufficient to account for the observed virus movements in the phloem, and the directional movements are opposed to this concept. For these reasons it may be assumed that cells other than parenchyma are the avenues through which the rapid movements take place. It seems probable that these cells are the sieve tubes.

A more extensive knowledge of the factors involved in the movement of organic substances in general through the phloem would undoubtedly provide a basis for a clearer concept of the possible path of viruses through this tissue. But if the speed and direction of movement of viruses are considered in connection with the theories that have been proposed to explain transport of organic materials through the phloem, the possibility must be considered that viruses may be released from the cytoplasm when they pass into sieve tubes and may occur more or less free in the lumen of the sieve tube. This concept is supported by the fact that the curly-top virus occurs in high concentrations in the phloem exudate from sliced surfaces of beets and also in the phloem content that moves from the phloem through intercellular spaces of cells of adjacent tissues and appears as drops on the surface of beet leaves and petioles. It is not probable that this exudate contains solid constituents from the phloem, and therefore the virus that the liquid exudate contains must have been free of the cytoplasm. In light of this evidence it seems probable that viruses occur free of cytoplasm in the phloem and that the chief path of rapid movement through phloem is the lumen of the sieve tube.

TISSUES IN RELATION TO SEED TRANSMISSION OF VIRUSES

Despite the fact that viruses are transmitted through the seeds of bean, wild cucumber, certain varieties of muskmelon, *Datura,* tobacco, lettuce, and potato, absence of seed transmission in general is very striking. This condition is not surprising with viruses that are restricted to the phloem. For, since there is no direct vascular connection between the embryo and the mother plant, the meristematic and parenchymatous tissue enveloping the embryo would function as an effective barrier to passage of virus in all stages of development of the embryo.

Lack of seed transmission of such viruses as that of tobacco mosaic, where invasion of the greater share of the living tissue occurs, is much more difficult to explain. Allard (1915) showed that the virus of tobacco mosaic passes into the ovule and occurs in both immature and mature seeds, but emphasized the fact that "A very efficient barrier guards against embryonic infection or the subsequent successful continuation of the disease from parent to seedling." Duggar (1930) found that ground seeds, especially those high in protein content, produced inactivation of the virus of tobacco mosaic but inactivation was never complete at the concentrations of proteins used. It was concluded that inactivation was not a factor of absolute protein content of the seed but was probably dependent on specific proteins or on specific compounds accompanying them. It was suggested that a probable relationship exists between lack of seed transmission and adsorption and inactivation of the virus by stored proteins in the seed. Lack of seed transmission under these conditions might involve entrance of the virus into the seed followed by inactivation by storage compounds.

Other evidence indicates, however, that lack of seed transmission may not depend on inactivation by the seed but on inability of virus to enter or remain active in such structures as microspores, megaspores, embryo sacs, and embryos. Much evidence points to the conclusion that seed transmission is determined at the time pollen and ovules are developing.

Nelson (1932) states that seeds from plants infected after flowering rarely transmit the virus of bean mosaic, indicating that virus transmission is determined in the very early stages of seed development.

Blakeslee (1921), working with the "Q" disease of *Datura,* found that diseased plants fertilized by pollen from healthy plants and healthy plants fertilized by pollen from diseased plants, produced seeds that transmitted the virus to the next generation, indicating the presence of virus in both pollen and ovules.

Reddick (1931) presented evidence indicating that the virus of bean mosaic is transmitted through the pollen of bean. Nelson and Down (1933), through cross-pollenation studies in bean, found that about 25 per cent of the pollen grains from infected plants and about the same number of ovules from infected plants, carried the bean mosaic virus.

The yellow and green ringspot viruses of tobacco have been shown by Valleau (1932, 1939) to be transmissible in the seed. Each of these viruses causes varying degrees of pollen sterility and gives evidence of being present in the pollen. Valleau (1932) suggested that if a virus enters the pollen it may also enter the embryo sac and that, therefore, pollen deformities produced by viruses may have some significance in indicating probable seed transmission.

Reddick (1936) presented evidence suggesting that the seed-borne virus of acropetal necrosis of potato may be carried in the pollen of infected potato plants.

In reasonably extensive tests[2] no virus was obtained from pollen taken from beet plants infected with beet mosaic nor from pollen from Turkish tobacco plants infected with common tobacco mosaic. Neither virus is seed transmitted.

Further studies are needed to determine the relation of other viruses to pollen and to ovules of susceptible species of plants. It is significant, however, that there appears to be no definite record of pollen infection by a virus not seed-borne. Conversely, where investigations have been reported, viruses that are seed transmitted show evidence of being present in pollen. If this apparent correlation between pollen infection and seed transmission has a general application, as the somewhat limited evidence indicates, pollen infection may be an index to susceptibility of gametes and may point to

[2] Unpublished data.

the critical period of development during which seed transmission is determined.

The factors governing seed transmission of viruses may reside in certain tissues that give rise to the male and female gametophytes or in the gametophytes themselves. Thus the mechanism which in many plants affords protection to the succeeding generation probably is closely associated with the reproductive processes. This protective mechanism is not clearly evident but enough information is available to afford a basis for further speculation regarding its nature.

If virus is absent from meristematic tissues the megaspore mother cell would be expected to be free of virus as would the resulting megaspores. In the degeneration of three megaspores and the enlarging of the fourth to form the embryo sac, it is possible that crushing or degeneration of cells immediately surrounding the megaspore may destroy protoplasmic connections between embryo sac and adjacent cells. If protoplasmic connections are essential for movement of viruses from cell to cell, as seems probable, destruction of these connections would tend to prevent infection of the megagametophyte.

The escape of pollen grains from infection, however, could not be explained on this basis since four pollen grains develop from each mother cell and there is no evidence that breaking of protoplasmic connections between cells would take place in the early stages of pollen differentiation. However, it seems within the limits of possibility that differentiation and maturation may be so rapid that pollen grains may separate from the mother plant and from each other often before viruses have a full opportunity to enter them.

A second point worthy of consideration in this connection is the fact that the microspores and megaspores are structures of the gametophytic generation which may conceivably modify their reaction to viruses. Factors associated with the production of sporophytic and gametophytic generations are capable of producing enormous morphological changes, as witnessed by the form differences between the sporophytic and gametophytic generations of plants of such groups as liverworts, mosses, and ferns. Morphologic differences of these magnitudes must be the result of distinctly different chemical constitutions and physiological functionings.

It seems logical to expect that in addition to the morphologic differences exhibited by individuals of the two generations, other differences equally marked would result and that some of these differences might well involve susceptibility to attack by viruses and parasitic organisms. The gametophytic generation of higher plants may contain protoplasm essentially different from that of sporophytic generation in respect to its resistance to invasion by viruses. Therefore, invasion of the gametophytic generation of a plant by a virus present in the sporophytic generation would be somewhat a matter of chance and on this basis probably would not be expected to occur oftener than infection of plants selected at random from the general population of species of the groups of plants attacked by the particular virus in question.

If a virus failed to enter the megagametophyte or microgametophyte for these reasons, or for other reasons, fertilization would initiate a virus-free embryo in a virus-free medium. The embryo, as a rule, develops rapidly, and it seems doubtful as pointed out by Caldwell (1934) and by Sheffield (1936) that there are any protoplasmic connections between the young embryo and the adjacent cells in any stage of embryonic development. The rapid elongation of the embryo in most instances would tend to break protoplasmic connections with adjacent cells if any were formed. In the absence of such connections it does not seem probable that infection of the embryo by passage of virus from adjacent cells into the embryo would be likely to occur even with viruses that are capable of reaching appreciable concentrations in the tissues immediately surrounding the embryo.

On the other hand, if a virus were able to enter the embryo sac or the microgametophyte and remain active, fertilization would result in the initiation of an embryo in a medium containing virus. The virus would be expected to remain in the cytoplasm of the zygote and its derivatives when cell walls were laid down, and would pass on to other cells in the succeeding cell divisions in the growth and development of the embryo, and become seed-transmitted.

The improbability of embryo infection by direct passage of virus from adjacent cells together with the apparent correlation between pollen infection and seed transmission in the instances cited, indicate that seed transmission may hinge on ability of virus to enter the megaspores, microspore or embryo sac and maintain itself in these structures through their successive developmental stages and through the developmental stages of the structure resulting from the fertilized egg.

CORRELATION BETWEEN VIRUS MOVEMENT AND FOOD TRANSLOCATION

During the past few years considerable evidence has accumulated that indicates quite strongly that the movement of viruses through infected plants is closely correlated with transport of organic food materials. This evidence has recently been reviewed by Crafts (1939).

In some of the earlier work on this subject it was shown (Bennett, 1927) that the virus of leaf-curl of raspberry produced symptoms on the top of the inoculated cane and moved downward into the root system, but under normal conditions it did not produce symptoms on non-inoculated canes during the first season. It was induced to move into non-inoculated canes, however, by cutting them back or by removing their leaves. The spring following the season of inoculation, the virus moved into all of the canes not previously invaded.

Results of experiments in which non-inoculated canes were ringed at intervals before and after growth started in the spring, indicate that the virus moved into these canes when the lateral shoots were about 2 to 4 inches long. It was suggested that failure of the virus to move into non-inoculated canes during the season of inoculation was due to the inability of the virus to move counter to the direction of major transport of organic materials, and that movement into these canes the season following inoculation was associated with the movement of food reserves from the infected root system following depletion of the food reserves in the canes.

Studies on the movement of the virus of tobacco mosaic have given results from which conflicting conclusions have been reached.

Holmes (1931) found that from the point of introduction of the virus into a tobacco leaf, the virus moved more rapidly in the direction of the petiole than in the direction of the leaf periphery. In later work he (1932) found that shading inoculated and non-inoculated leaves induced changes in the path of movement that indicated some direct or indirect connection with the carbohydrate supply. However, since the virus was able to move from leaves starved with respect to carbohydrates it was suggested that the relation may be indirect.

In experiments with tomato, Samuel (1934) found that after being restricted to a limited area of the inoculated leaf for a period of 2 to 4 days following inoculation, the virus moved rapidly toward the root system, then upward to the top of the plant except in plants with fruit clusters, in which case it moved into the fruits first. Samuel suggested a direct correlation between food movement and virus movement, and postulated, on the basis of his evidence, that the metabolites from the leaves moved first to the roots and then to the tops. More recent work by Kunkel (1939) has shown, however, that when the virus of tobacco mosaic passes from an inoculated leaf into the stem of a tomato plant, movement is not always first in the direction of the roots but may be either upward or downward or in both directions from the point of entrance.

Grainger (1933), by inoculating tobacco leaves at the distal ends and severing them at different distances from the point of inoculation at different time intervals, obtained results which he interpreted as indicating that the virus of tobacco mosaic moved at a uniformly accelerated rate. He concluded that movement was through the ground tissue and that it was associated with multiplication of the virus and unrelated to food translocation.

Caldwell (1934) found that the virus of tobacco mosaic passed out of leaves that were inoculated and placed immediately in the dark, and that the virus showed no greater tendency to enter mature leaves in the dark than it did to enter mature leaves in the light. Movements in the stems were in two directions. He (1936) found also that the virus was able to move out of immature leaves. Caldwell (1934) concluded that virus movement is independent of movement of food materials and that under certain conditions movement was apparently in a direction opposite that of the metabolites.

However, the results obtained by Grainger and Caldwell are capable of interpretations that do not necessarily conflict with the concept of a correlated virus movement and food translocation. As Caldwell pointed out, tobacco leaves wilt and die after they have been in the dark for about ten days. It is possible in such leaves that the directional movements of organic materials are not greatly different from those in normal leaves, due first to an outward movement of reserves and later to outward movement of products of protoplasmic disintegration.

Movement of virus out of immature leaves may be correlated with outward movement of organic materials during periods favorable for rapid carbohydrate synthesis. It is of interest in this connection that Holmes (1932) found a greater movement of virus toward the periphery in immature leaves than in mature leaves and considers that "The reversal of the direction of movement of virus in leaves inoculated when very young seems to indicate a relation of some kind between the movement of food into a young and dependent leaf and the movement of virus, and suggests that virus moves toward the periphery until the leaf reaches a degree of maturity which allows it to export some food material to dependent growing parts."

Although it has been shown repeatedly that the virus of tobacco mosaic moves from an inoculated leaf to both the top and the roots in Turkish tobacco in a relatively short period, it has not been shown that movement in the two directions is simultaneous. Diurnal or other directional reversals of food movement would account for the observed virus movements.

In more recent work (Bennett, in press) additional evidence supporting the concept of a correlation between the movement of the tobacco mosaic virus and food translocation has been obtained. In vegetative plants of Turkish tobacco having a main stem in a horizontal position and a basal sucker in a vertical position, basipetal movement was rapid and acropetal movement was slow. The reverse was true in similar plants maturing seeds on the main stem. In vegetative plants, acropetal movement was accelerated by darkness and defoliation. Basipetal movement was very slow in main stems in the dark and in the majority of plants tested the virus failed to move out of darkened stems in 40 days; whereas in comparable stems in the light it moved out in all instances in 4 days or less.

In plants of *Nicotiana glauca* having top and basal grafts of Turkish tobacco separated by 3 feet of stem, virus moved from the top graft to the basal graft and produced symptoms in 6 to 9 days. Movement in the opposite direction, presumably counter to the direction of major food transport, was very slow and in some instances the virus did not move a distance of 3 feet acropetally in periods of 224 to 253 days.

McClean (1931) states that the virus of bunchy-top of tomato is restricted to leaves partially developed at the time of infection or developed subsequent to infection. When only the roots of tomato plants were inoculated no symptoms developed on the top. It is possible that this latter result may be due to inability of the virus to move out of the roots against a food gradient, rather than inability of the virus to produce infection through root tissue.

It is probable that certain parts of root systems of plants may be able to localize viruses for considerable periods under normal conditions of growth. Mulvania (1930) found that when roots of tobacco plants were inoculated with the virus of tobacco mosaic, symptoms did not appear on the tops of the inoculated plants. Bennett (in press) obtained similar results but found that the roots were susceptible to infection and that the virus could be induced to move out of the root system by removing the tops.

Kunkel (1930) found that when peach buds from yellows trees were placed in vigorous young peach trees some distance above the ground level symptoms of yellows developed in a minimum time of six weeks; whereas when the infected buds were placed at or near the ground level the incubation period of the disease often extended over many months. This was found to be due to the fact that the virus moves quickly down but rather slowly up the peach stem. Downward movement was estimated to be about 10 times faster than upward movement. On a basis of a correlated virus and food movement these results would be expected except perhaps when the trees were producing rapid top growth immediately following a period of dormancy.

In studies (Bennett, 1937) on the movement of the virus of curly-top of beet it was shown that the virus moved downward from grafts of Turkish tobacco through stems of *Nicotiana glauca,* much more rapidly than it moved in the opposite direction. Defoliation of the tops, however, stimulated rapid movement upward.

Using the same virus it was found that in beets with three shoots on a common root system, the virus was retained in an inoculated shoot for periods as long as six months with no appearance of virus in non-inoculated shoots during this time. However, defoliation of one of the non-inoculated shoots resulted in movement of virus into it and the production of symptoms in a period of a few days. Also, when one of the non-inoculated shoots was placed in the dark it soon became diseased. A repetition of this experiment using the virus of beet mosaic gave similar results.

A relatively high concentration of curly-top virus was found (Bennett and Esau, 1936) in seeds from infected plants, perhaps indicating a movement of virus into seeds with food materials, resulting in its accumulation as a residue.

The virus moved inward from the point of inoculation at the tip of a green leaf of sugar beet a distance of 6 inches in 6 minutes, whereas in etiolated leaves in the dark it failed in most instances to move out of the inoculated leaf in periods of 7, 14, and 21 days. These results were interpreted as indicating, not only that there is a correlation between virus movement and food translocation, but also that virus movement is dependent largely on the same agencies that are responsible for food translocation.

The available evidence favoring the view that virus movement is correlated with translocation of organic solutes seems extensive enough to justify serious consideration of the possibilities of using viruses as indicator materials in the study of the general subject of translocation in plants. If viruses may be used for this purpose they have the following distinct advantages: (1) If viruses are high molecular weight proteins and if they are produced from normal plant proteins as suggested by Stanley (1936), they may not be greatly different in many basic chemical and physical properties from certain normal protein constituents of the plant. Movement, therefore, would be expected to closely parallel that of the parent proteins. (2) Viruses may be introduced into epidermal cells by rubbing inoculum over the surface of leaves, or directly into the phloem by utilizing a suitable insect vector. Thus by selection of the virus and the method of introducing it into the plant, movement in either ground tissue or phloem may be studied. (3) Viruses move and remain active in a very large number of plants, giving a wide range of species and varieties for study. (4) The extent of spread from points of introduction may be determined by several different methods some of which permit a high degree of accuracy of measurement.

The objection has been raised (Curtis, 1935) that since viruses cause phloem necrosis and other abnormalities their movement may be abnormal and unrelated to normal solute movement. It may be pointed out, however, that the majority of viruses cause no detectable injury to parts that are mature at the time of virus introduction. It is in such parts that virus movement can best be studied. Moreover, few of the viruses that cause mottling produce detectable injury to the phloem, and it is not difficult to find viruses that cause no recognizable symptoms on certain species and varieties of plants. The pathologic effects, therefore, would not seem to offer any great difficulties in the use of viruses as indicators of movement.

MECHANICS OF VIRUS MOVEMENT

It seems highly improbable that viruses possess any autonomous means of locomotion, since virus particles are known to be very small and much evidence indicates that they may be of molecular proportions. Movement, therefore, must result from the operation of ordinary physical and chemical forces common to the plants in which the viruses occur.

Evidence indicates that viruses are subjected to forces that produce two distinctly different types of movement, as indicated by the respective rates of travel. One set of factors gives rise to a relatively slow movement through parenchyma, and a second set of factors gives rise to rapid movement through the phloem. These two types of travel may be considered separately.

Movement in parenchyma. Uppal (1934) made measurements of the spread of the virus of tobacco mosaic from the upper epidermis to the lower epidermis in leaves of *Nicotiana sylvestris* and found a movement of 7 to 8 microns per hour. For a number of other viruses the rate of movement through parenchyma can be rather accurately estimated by measuring the rate of radial spread of local lesions. Measurements of this type with tobacco mosaic, beet mosaic, tomato spotted-wilt and bean mosaic have given a relatively uniform result and have indicated a radial spread of less than 1 to about 2 millimeters per day. Other evidence indicates that spread of viruses in general through parenchyma is of this order. Therefore, factors responsible for this movement probably need not account for movements much in excess of 2 millimeters per day.

In the movement of the virus of common tobacco mosaic from cell to cell, Stanley (1936) suggested that active virus protein in one cell may catalyze the production of virus protein in adjacent cells without actually passing out of the cell. Under such conditions movement of virus from cell to cell would not necessarily occur but would only appear to take place due to the catalytic action of virus protein at the periphery of the protoplasm of one cell on the precursor protein in adjacent cells.

However, it does not seem necessary to invoke the operation of such a mechanism in explaining movement from cell to cell. Introduction of the virus into a living parenchyma cell would be followed by distribution of the virus to all parts of the cell by protoplasmic streaming and diffusion. The virus would readily pass into adjacent cells through the plasmodesmata by means of diffusion, perhaps aided by protoplasmic movement. Multiplication of virus probably would influence the rate of spread by diffusion to a certain extent, but it would be expected that maximum concentration would soon result at the point of introduction and that on the advancing margins of virus invasion a state of equilibrium would be reached in respect to concentration and diffusion and that soon after introduction the virus would reach a state in which it would move at a uniform rate, in so far as rate of movement was affected by diffusion, and not at a uniformly accelerated rate as has been suggested for certain viruses.

Movement in phloem. The rapid rates of movement of viruses in phloem (as high as 1 inch per minute) indicate that protoplasmic streaming and the ordinary rates of diffusion may be ignored as major factors in accounting for this movement. The apparent correlation with food translocation indicates that movement through the phloem may be dependent on normal processes that function in plants in the transport of food materials. For this reason it may be well to consider some of the mechanisms that have been proposed to explain the transport of various kinds of substances through plants, in connection with the probable mechanism responsible for virus movement.

The rates of movement of the viruses of streak of maize (.3 cm. per minute) and of curly-top of sugar beet (2.5 cm. per minute) are so great that they may suggest a movement similar to that of a stimulus. These rates are more or less of the same order, however, as those calculated for movement of sugars through the phloem of potato, cucurbits (Crafts, 1931, 1932), and cotton (Mason and Maskell, 1928). These results with the movement of sugars demonstrate that the plant possesses a mechanism capable of effecting rapid transport of elaborated materials. There seems no logical reason, therefore, for assuming that virus invasion of the phloem network is not brought about chiefly by actual transport of virus units.

Went (1932) suggested that growth hormones move along electrical potentials toward rapidly growing regions. It has been shown that, in general, virus entities, or the particles to which they are attached, carry a negative charge. It is conceivable that a positive potential might cause rapid movement of virus particles under certain conditions. However, to accept this theory of virus movement certain assumptions regarding conditions and changes in plants must be made. For example, it would be necessary to assume that the potential is much less effective in causing movement in parenchyma than in phloem. In the case of the virus of curly-top it would be necessary to assume that the potential gradient is basipetal in stems of *Nicotiana glauca* and in the beet leaf and that defoliation or darkness is capable of reversing this gradient.

Van den Honert (1932) called attention to the possibility of movement of materials along the interfaces of protoplasmic material in the phloem due to surface tension forces. Possibly such a mechanism would provide for the rates of virus movement observed if viruses are capable of moving as monomolecular films along the surface of protoplasmic layers, but it would not provide for the directional movements observed. In this system substances would move independently and equally in all directions in which there were paths for movement. Therefore, viruses would move against food gradients as fast as they move with food gradients, and defoliation and darkness should have no effect on rate of invasion of a plant part, all of which is opposed to the observed facts.

One of the oldest theories of food movement invokes diffusion and protoplasmic streaming to bring about transport. Curtis (1935) has reemphasized this theory with the suggestion that protoplasmic streaming in the sieve tubes may occur and possibly may be much faster than heretofore suspected. However, even if streaming of protoplasm in the sieve tube actually occurs, it does not seem probable that it can be fast enough to provide for the more rapid rates of virus movement, and so far as viruses are concerned the theory has the added weakness that it falls far short of accounting for the directional movements observed.

Münch (1930), in a theory that was later discussed and modified by Crafts (1931), proposed that there is a pressure flow of liquid materials through the phloem from supplying to receiving cells. According to this concept, sugars pass from the synthesizing cells into the phloem where an increase in osmotic pressure is produced resulting in a higher hydrostatic pressure. This causes a movement of materials through the phloem in the direction of regions having lower hydrostatic pressures. The osmotic pressure is lowered in regions of utilization and storage by removal of sugars from the phloem. This results in loss of water by the phloem and in reduction of hydrostatic pressure in these regions. Movement, therefore, toward regions of utilization and storage would be continuous.

The major objections to this theory of transport are summarized by Curtis (1935). He suggested that perhaps the exudate which is obtained from the cut ends of cucurbit stems and which has been assumed to flow from the phloem, does not originate in the phloem and therefore cannot be accepted as evidence of flow through the phloem of the uninjured plant. He suggested further that the theory of pressure flow as modified by Crafts rests on at least three unproved assumptions: (1) that the supplying cells can in some way introduce sugars into the phloem in such a manner that they will develop a pressure gradient leading to the receiving cells, (2) that the sieve tubes are completely permeable and offer a minimum of resistance to the flow of solutions through the lumina and cross sections walls at all points and yet that the phloem is so enclosed by cambium and phellogen as to prevent leakage, and (3) that receiving cells can absorb sugars against a gradient with such rapidity as to lower greatly the concentration within the walls outside the living membrane. The general theory of mass flow of solutes in the phloem is criticised because it does not allow for simultaneous movement of materials in two directions.

Upon critical analysis, however, and in the light of a certain amount of additional evidence, these objections do not seem to constitute insurmountable obstacles to the acceptance of the general principles of the theory of pressure flow.

Further work by Crafts (1936) furnishes strong support for the contention that the exudate obtained from the cut stems of cucurbits is derived directly from the phloem. Recently Crafts (1939) demonstrated exudation from the cut surfaces of plants of *Macrocystis* and states that this exudate unquestionably came from the phloem, since there is no xylem in *Macrocystis* and the sieve tubes of the phloem are the only specialized elements capable of rapid conduction. It was stated also that by using suitable technique phloem exudation can be demonstrated quantitatively in most woody species.

These results seem to show rather conclusively that liquids are able to flow through cross-sectional areas of the phloem under a positive pressure.

Phillis and Mason (1933) showed (as Curtis noted) that the phloem of the cotton plant is able to accumulate sugars against a gradient. This suggests at least that a mechanism may exist in the plant for establishing pressure gradients in the phloem.

Whether the resistance which the phloem offers to mass flow of materials is prohibitive remains to be determined. An earlier idea (Crafts, 1931) that movement takes place chiefly through the cell walls of the sieve tubes seems untenable, as pointed out by Steward and Priestley (1932), because of the high pressures required. However, calculations by Crafts (1933) indicate that if the sieve tube lumina are the chief channels of transport the pressure required may not be excessive. The degree to which the cell layers incasing the phloem are able to preserve a positive pressure in the phloem cannot be stated with certainty, but the mere fact that positive pressures exist in the phloem shows that the encasing layers are able to function with at least a certain degree of effectiveness in this respect.

The rapidity with which receiving cells can absorb sugar from the phloem and thus steepen the sugar gradient in the phloem undoubtedly varies considerably with conditions. It is evident that such plants as sugar beet, sugar cane, and date are able to remove sugar from the phloem and accumulate it in storage cells against steep sugar gradients. The rate of storage in some of these plants shows that movement from the phloem into the receiving cells is rapid.

It is quite obvious, of course, that the pressure flow concept does not permit of continuous simultaneous two-directional movement through the phloem. More evidence is needed as to the amount of two-directional movement necessary to satisfy the plant's distributional requirements, and as to the extent to which such movement actually occurs. The evidence for simultaneous two-directional movement is limited. Phillis and Mason (1936) found that nitrogen and carbohydrates moved simultaneously in opposite directions in the stems of cotton plants. Although they consider that in their experiments movement of both nitrogen and carbohydrates probably took place in the phloem, they point out that the possibility that the nitrogen moved in the xylem cannot be excluded. Palmquist (1936) presented evidence interpreted as indicating simultaneous movement of carbohydrates and fluorescein in opposite directions in the phloem of bean leaves. There is still some question, however, as to the tissue in which flourescein moves and also as to the factors involved.

It would seem that further effort should be directed toward determination of the possibilities for differential distribution of materials that may be effected by frequent directional reversals of movement of materials and toward determination of the extent of movement of materials in opposite directions in different vascular bundles of the same stem or leaf.

Much of the evidence on virus movement strongly supports the concept of mass flow of liquid phloem content. This evidence clearly indicates that with certain viruses at least, movement is decidedly unidirectional from the point of introduction and movement is in the direction of major transport of elaborated food materials. The evidence for correlation between virus movement and food transport has already been summarized. It seems to clearly indicate that viruses move rapidly in directions of food utilization and storage, and slowly in opposite directions. A correlation of this kind would indicate some type of flow through the phloem.

Virus movements that may be interpreted as furnishing doubtful support to the pressure flow concept involve a type of possible two-directional movement and apparent differences in rates of movement of two viruses introduced into the plant simultaneously.

Evidence for two-directional movement of tobacco mosaic in tomato was presented by Kunkel (1939). In these experiments the virus was allowed to enter the stem of tomato plant through the petiole of an inoculated leaf located approximately midway between the top and roots. In a two-hour period the virus moved only to the tops in some plants, only to the roots in other plants, and to both tops and roots in still other plants.

Kunkel considers that the virus moved simultaneously in two directions in the stem in some of these plants, but suggests that movement in the two directions may have been in different vascular bundles. It would seem possible to have such a movement also in the same vascular bundle under certain conditions, if virus movement is influenced by food translocation. In a rapidly growing tomato plant carbohydrates are being used by the top and by the roots. These carbohydrates are supplied by leaves located along the total length of the stem. Assuming demand by both top and roots there must be a portion of the stem in which movement is toward the root and a portion in which movement is toward the top. This would provide a stem zone out of which movement would be in two directions. The position of this zone would be expected to fluctuate with varying demand for food by top and roots. If virus were being introduced into this zone it might, in some cases, move in both directions from the point of entrance, and movement might be in one or more vascular strands.

The failure of the virus, moving at a maximum rate of 7 inches per hour in these tests, to move toward the root in certain plants in periods of 12 to 28 hours is decidedly opposed to the concept that the factors responsible for movement operate continuously to cause simultaneous movement in two directions in all parts of the stem.

Differences in the rate of invasion of plants by the separate components of a virus mixture have been found in certain instances. Smith (1931) found that when the "X" and "Y' viruses of potato are introduced into a leaf by mechanical inoculation, the "Y" virus appears in the young leaves of the inoculated plants about 2 days ahead of the "X" virus. Curtis (1935) pointed out that this seems opposed to the concept of unidirectional flow of phloem content. As Samuel (1934) has emphasized, however, it has not been demonstrated that two such viruses actually move at different rates in the phloem but only that they arrive at points of test at different times. This difference in time of arrival at points of test may result from failure of two viruses introduced simultaneously into the epidermal cells of leaves, to move at the same rate through intervening parenchyma cells and to enter the phloem at the same time. Unfortunately no results are available as to the relative rates of movement of viruses introduced simultaneously into the phloem instead of parenchyma.

It is recognized, however, that even if two viruses were introduced simultaneously into the same sieve tube they might move at different rates in a flowing medium if they differed in size or shape. There is considerable evidence indicating that the pores in the sieve plates are, in some plants at least, extremely small and filled with slime.

If materials move through these pores, or if they move partially through the cell walls as suggested by Crafts (1932), particles of different shapes and sizes may encounter different degrees of resistance to movement. Other things being equal, small spherical particles would encounter less resistance in passing through such obstuctions than would be met by larger spherical particles or by elongated particles. Rate of movement, therefore, may be influenced to a certain extent by the size and shape of the virus particles and possibly also by their electrical charge.

Differential travel of components of virus mixtures from plant roots toward the tops of actively growing shoots has been found in certain instances and probably occurs with a number of virus combinations.

For example, when plants of certain susceptible varieties of raspberry were inoculated with the viruses of leaf-curl and red raspberry mosaic, simultaneously, it seems quite certain that the latter virus invaded the non-inoculated canes of the plant more rapidly than the same canes were invaded by the leaf-curl virus. When a cane of a black raspberry plant was inoculated with the viruses of yellow mosaic and red raspberry mosaic, the virus of red raspberry mosaic moved into the non-inoculated canes of the plant during the first season; whereas, the virus of yellow mosaic did not move into canes until the second season.

When a shoot of a beet plant having three shoots was inoculated (Bennett, 1938) with the viruses of mosaic and curly-top the virus of mosaic, as a rule, moved into the non-inoculated shoots several days or even weeks before the presence of the virus of curly-top could be demonstrated in such shoots.

It has not been shown in any of these experiments that the downward movement in the phloem from the points of introduction and in the direction of major food movement, was different for the components of the virus-combinations involved, but only that upward movement into non-inoculated canes or shoots, counter to the direction of major food movement, was different.

When the possible tissue relationships of these viruses are taken into consideration this evidence does not necessarily conflict with the idea that mass movement of materials in the phloem is important in the movement of materials throughout the plant. There is strong indication that the viruses of leaf-curl and curly-top are closely restricted to the phloem and there is some evidence also that this may be true of the virus of yellow mosaic of raspberry. The viruses of red raspberry mosaic and of beet mosaic probably occur in both parenchyma and phloem. A differential movement of the type observed might be expected with two viruses having these two tissue relationships.

If they were introduced into the phloem of a leaf or the tip of a cane, simultaneously, both would move toward the root system at the same rate if they were carried by mass flow of phloem content, assuming no selective interference with movement. After entering the crown or root the virus restricted to the phloem would move more slowly upward through mature parts against a downward flow of materials and at times its movement backward due to mass flow might be faster than upward movement due to the operation of other factors.

The virus able to move and multiply in both parenchyma and phloem might effect much of its upward movement in parenchyma tissue. This movement would be relatively slow. Also, due to temporary changes in food relations, it might be carried upward by surges of materials from the roots, if liquids in the phloem move under a pressure gradient. Such movements would be expected to carry both types of virus upward in the phloem for the distance of the movement. The phloem-limited virus would remain in the phloem and tend to be carried back when the direction of movement of materials was reversed. The mosaic virus would be expected to pass out of the phloem into adjacent parenchyma tissue and become established there. From this newly invaded region the virus would not only continue to travel through parenchyma, but it would also multiply and furnish a more or less permanent source of virus to the phloem at the new levels of invasion. Each successive upward movement of materials in the phloem would elevate the virus to higher levels in the non-inoculated part.

It is easily conceivable that in this manner, two viruses with the indicated tissue relationships, could readily be separated, one passing into non-inoculated parts more rapidly than the other even though the two viruses were introduced into the phloem of the plant at the same point, simultaneously.

In this connection it is a rather significant fact that viruses such as the virus of red raspberry mosaic and the virus of beet mosaic, that evidently occur in both phloem and parenchyma, travel in directions opposite major food movement at greater rates than viruses such as the virus of leaf-curl of raspberry and the virus of curly-top of sugar beet, that appear to be more or less restricted to the phloem. Rates of travel of viruses of the first types in directions opposite major food movements, however, are far below those determined for movement in the directions of major food movement.

Definite conclusions at this time regarding the mechanism responsible for virus movement through the phloem would be premature. From the evidence indicating a correlation between virus movement and food transport it may be suspected, however, that movements of both viruses and food materials are brought about by the same basic physiological processes. If this is true, the observed virus movements are opposed to the concepts of transport by diffusion and protoplasmic streaming and movement along protoplasmic interfaces but they harmonize reasonably well with the concept of pressure flow of liquid phloem content.

The existence of a plant mechanism capable of bringing about a pressure flow of liquid phloem content is still a matter of controversy among plant physiologists. It may be said, however, that if such a mechanism does operate it would satisfy most of the requirements of virus movements that have been determined. It follows, therefore, that the mechanism responsible for virus movement must be able to effect movements essentially similar to those that would be expected to result from pressure flow of liquid phloem content. For this reason the pressure-flow concept may be helpful to those interested in virus movement. On this basis a logical picture of invasion of plants by viruses may be drawn which conforms in all essential details with the virus movements that have been observed.

Under such a concept, the introduction of a mechanically transmissible virus into the epidermal cells of a leaf would be followed by increase, and distribution would be effected by protoplasmic streaming and diffusion perhaps aided by the processes responsible for virus increase. The virus would pass into adjoining cells, probably through plasmodesmata, and be distributed by the same processes that were responsible for distribution of virus in the inoculated cell. Eventually the virus would pass into the phloem through the protoplasmic connections and come in contact with a stream of material moving more or less rapidly toward regions of food utilization or storage. Phloem-limited viruses would be introduced directly into the phloem content through the agency of insect vectors.

Viruses would tend not to move into mature leaves and regions supplying an excess of photosynthates. A reversal of food requirements in any region would cause a reversal of flow in the phloem, and virus would be carried passively in the direction of the new movement. In plants in which food movement can be modified and to a considerable extent controlled by defoliation and darkness, virus movement could be correspondingly influenced. This would explain the rapid movement of the curly-top virus into defoliated or darkened shoots of beet when corresponding shoots not defoliated or darkened remained free of virus for relatively long periods. It would account also for the apparent tendency of the virus of tobacco mosaic to move toward fruiting parts in tobacco and tomato and for the relatively high concentrations of curly-top virus found in beet seeds from infected plants. Etiolated leaves or shoots receiving food from regions of supply would be expected to retain virus as they have been observed to do in beet and tobacco.

SUMMARY AND CONCLUSIONS

In their increase and movement in infected plants viruses appear to be limited to living tissues. However, there is evidence that different types of living tissue offer varying amounts of resistance to virus invasion, depending on the plant affected, on the virus involved, and on the environmental conditions to which the infected plant is subjected.

The principal kinds of tissues known to be susceptible to virus invasion are the phloem and the various types of parenchyma. All types of meristematic tissue seem to be resistant. In some instances there is evidence that cell invasion at growing points may not occur until differentiation into parenchyma begins. If viruses occur in meristem, they apparently cause no direct injury to this type of tissue.

On the basis of their ability to invade parenchyma and phloem, viruses exhibit three more or less clearly defined relationships to tissues: (1) restriction to parenchyma, (2) close association with the phloem, and (3) occurrence in both phloem and parenchyma.

Viruses that are restricted to parenchyma are less common than those that show other relationships to tissues. This would be expected since such viruses would be less readily disseminated.

However, evidence suggests that the virus of phony disease of peach may be limited to parenchyma of the woody cylinder and it seems probable that the virus of tobacco necrosis may prove to be limited to parenchyma under most conditions. Certain normally systemic viruses are evidently limited to parenchyma in plants where they produce only local lesions.

Several viruses that appear to be rather closely restricted to phloem are known. Leaf-curl of raspberry and curly-top of sugar beet are considered representative diseases caused by viruses of this type. Phloem-restricted viruses produce diseases characterized by disturbances arising mainly in the phloem. Typical symptoms are phloem necrosis, vein translucency and distortion, leaf distortion and rolling, and general stunting and discoloration of the affected plant. Infection by mechanical inoculation is difficult. Vectors are limited to insects that feed on the phloem and probably also to insects that are able to pass virus through their bodies and liberate it with the saliva in feeding.

Viruses that occur in both phloem and parenchyma are numerous, and probably include all of the viruses of the mosaic-producing type. Characteristic symptoms result from disturbances in the parenchyma, and consist chiefly of local lesions and mottling. Phloem disturbances are usually minor or absent. Often infection by mechanical inoculation is easily accomplished. In general, insect vectors are more numerous and tend to lack specificity. Phloem-feeding is not essential to transmission.

The measured rates of virus movement following introduction into the plant vary from one-tenth of a centimeter per hour for the virus of tomato mosaic in tomato, to 152.4 centimeters per hour for the virus of curly-top in sugar beet. Rate of movement is influenced by the plant in which movement takes place. For example, the virus of curly-top moves at a much greater speed in sugar beet than in tobacco. The extent to which different viruses may move at different rates in the same plant remains to be determined. Evidence available at present indicates that certain factors operating in the plant may be more important than the virus in determining rate of movement.

The path of virus movement appears to be the living cell. Apparently viruses are unable to move through the cellulose structure of the cell wall. Limited evidence suggests that movement through parenchyma takes place in the cytoplasm. Plasmodesmata probably serve as avenues of passage from cell to cell. When viruses enter the vascular elements there is indication that they are released into the liquid content of the phloem and it seems probable that the chief path of movement through the phloem is the lumen of the sieve tube.

In the absence of vascular connections between the embryo and the mother plant, restriction of a virus to the phloem would prevent seed transmission. Freedom from seed transmission of viruses that occur abundantly in parenchyma is more difficult to explain. It seems evident, however, that the factors involved are associated with the mechanism of reproduction. Possibly protection to the succeeding generation may be afforded by inability of viruses to enter microspores and megaspores and maintain themselves throughout the subsequent developmental stages of these structures and their derivatives. The fact that pollen infection is associated with all cases of seed transmission that have been investigated adequately, and that no virus not transmitted in the seed is known to occur in pollen, lends support to this hypothesis.

Two possible explanations may be advanced to account for failure of viruses to enter microspores and megaspores: (1) Destruction of protoplasmic connections between sporophytic tissue and the microspores and megaspores may take place before viruses have entered the latter structures; (2) The gametophytic generation may in many cases possess immunity to infection due to physiological characteristics resulting from its gametophytic constitution.

If virus failed to enter the gametophytic structures, fertilization would be expected to initiate an embryo in a virus-free medium. Since there is no evidence of plasmodesmatal connections between the embryo and adjacent cells, the embryo would be expected to remain free of virus even though adjacent tissue might be infected.

Certain viruses have been shown to move more rapidly in directions of regions of food utilization and storage than in opposite directions. The direction of major virus movement may be partially controlled by influencing the direction of food transport by such measures as reducing the leaf surface on appropriate plant parts or by subjecting the parts to prolonged periods of darkness. Rather extensive evidence supports the concept that virus movement is definitely correlated with the normal transport of organic food materials.

Factors responsible for invasion of plants by viruses must provide for two distinct types of movement. The first is a slow movement through parenchymatous tissues and the second is a much more rapid movement through the phloem.

Movement through parenchyma is probably effected through protoplasmic streaming and diffusion possibly aided by the processes responsible for virus increase.

The factors responsible for movement of viruses in the phloem are of considerable interest because of the speed and directions of movement in this type of tissue. These factors must provide for movements as great as 2.5 cm. per minute and for a type of undirectional movement which seems to be correlated with transport of photosynthates. It seems probable on the basis of rate and direction of observed virus movements, that diffusion, protoplasmic streaming, forces operating to promote movement along protoplasmic interfaces, and possibly electrical potentials, may be ruled out as major factors in the movement of viruses in the phloem. In light of present knowledge it seems probable that the mechanism responsible for virus transport in the phloem is able to effect movements essentially similar to those that would be expected to result if a pressure-flow mechanism, such as that proposed by Münch (1930), were operating in the transport of elaborated food materials.

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