INTRODUCTION

The cultivated tomato (Lycopersicon esculentum) is subject to a range of diseases resulting from infection with certain strains of tobacco mosaic virus (TMV). The mosaic disease in tomato was described in the Netherlands in 1910 (158), and in the USA in 1916 (9). Since then considerable efforts have been made to understand its epidemiology and to devise control measures. The fact that the disease remains widespread suggests that the efforts to date have not been entirely successful.

TMV is the most infectious plant virus known; young tomato plants are infected when rubbed with infected tomato sap diluted one part in 5 million parts of water (27). It is also the most persistent virus in terms of its ability to survive outside plant cells and in dead tissues (38). Its numerous strains infect a very wide range of plant species, although there is considerable specificity between strain and host. It is not surprising, therefore, that the disease is ubiquitous wherever tomatoes are grown under glass and in many areas of outdoor cultivation. Tomatoes are not a cheap crop to produce, particularly under glass, and until recently about 20% of world production probably was lost because of TMV. The literature on TMV is voluminous; so also is the literature on the disease in tomato. Many of the papers mentioned in my 1960 review (25) are omitted from this paper.

THE DISEASE IN TOMATO

Causal Viruses

Early investigators reported that ordinary or mild tomato mosaic virus was similar to common TMV. In tobacco of the White Burley type, TMV was known to develop systemically but did not cause necrotic local lesions (2, 9). In tomato,
TMV caused a light and dark green mottle, slight plant stunting, and sometimes leaf distortion. Since then many serologically related strains of TMV have been isolated from tomatoes, and the problem is to decide what was meant by tomato mosaic. By 1927 stripe or streak disease of tomato was often associated with "mosaic," plants occasionally developing necrotic areas on leaves, brown necrotic stripes on the stems and petioles, and sometimes brown pits on the fruits (15). This disease was called also single virus streak to distinguish it from that caused by a mixture of TMV and potato virus X.

Inoculation of tomato with sap from stripe plants often caused mosaic only (1) and in 1940 it was demonstrated that the strain of TMV common in German tomato crops was not common tobacco virus, but caused only necrotic local lesions when inoculated into certain *Nicotiana* species (97). Occasionally the common tobacco strain occurred as well, causing a systemic infection. Experimentally, it is difficult to reproduce and distinguish single virus streak from double virus streak—the symptoms may result from a complex of factors, including environmental shocks, root damage, and possibly a mixture of established or unstable tomato strains of TMV (124). New strains of TMV with increased infectivity, or extended host range, or both have resulted from passage through resistant hosts. This suggests that protracted periods of evolution are not required for changes in strains (50, 91, 92).

A review of the TMV strains and isolates would require a paper by itself. The literature is confused because different workers have used symptoms to distinguish strains, or indicator plant reactions, host range, genetic or biochemical techniques. A paper on this subject by M. Hollings is to be published in 1976.

Tomato mosaic is now known to be caused by a range of strains, allied to those causing streak, most of which cause local lesions only when inoculated into appropriate test plants, such as the necrotic line of White Burley tobacco selected by workers in the Netherlands (143). Unfortunately the reactions of such test plants are not entirely consistent, and several tomato mosaic serotypes invade them systemically. Nevertheless, surveys of commercial tomato crops in several countries, using different indicator plants such as the necrotic strain of White Burley tobacco, *Nicotiana sylvestris* and Petunia, have confirmed that tomato and tobacco crops tend to be infected by different viruses. In the UK during 1960–61 only 4 out of a total of 187 crops that were infected with the tomato strains of TMV carried a tobacco strain as well; these 4 consisted of grafted plants and the workers had been smoking while grafting (26). Other surveys have shown that the tomato strains prevail in Canada (56), Korea (17), Japan (83), Belgium (151), the Netherlands (124), and elsewhere, although Dutch, Korean, and Japanese workers found some tomatoes infected with tobacco strains of TMV. Even when tomatoes were grown contiguous to tobacco in Canada, the TMV strains infecting both crops were distinct (90). In the Netherlands, work based largely on symptom expression distinguished eight strains in tomato, of which the normal mosaic was the most common (124). The common tobacco strains were seldom found, and the other six were more closely related to the tomato than to the tobacco type. The common tomato strain not only more easily infected tomato
than the tobacco strain but also multiplied faster after invasion (83). The two strains do not cross-protect and they can coexist in tomato (26, 93, 124).

**Symptoms**

The reaction of the tomato plant to infection with TMV depends upon cultivar, virus strain, time of infection, soil nutrient, water status, day length, light intensity, and temperature (55). In addition to the leaf mottle or mosaic, leaves tend to be elongated or otherwise distorted in appearance and smaller and more pale than those on healthy plants. Leaves of infected plants sometimes are scorched in sunlight, particularly soon after infection and after wetting with water, and lower leaves die earlier than those on healthy plants (28).

Infected seedlings are stunted, with smaller, less fibrous root systems as well as less foliage; late infection does not affect root growth significantly (32, 152). The smaller root systems in young plants are caused by a failure to grow, rather than by necrosis.

During the incubation period before symptoms show, growth slows considerably (4). There is a sharp drop in transpiration rate coincident with the appearance of symptoms but then the rate increases gradually, and usually TMV-infected leaves transpire faster than healthy ones. The water content of infected plants is lower than that of healthy ones during the early stages of disease, but later it is higher (68, 132). Although TMV can seriously affect quantities of root, shoot, and fruit produced, depending mainly on the age of the plant at the time of infection, it does not affect the general pattern of growth and development (32).

Leaf symptoms often become less severe as infected plants age. TMV concentration in young leaves, as well as symptom severity, increases with increasing temperature from 16 to 28°C, but once the maximum concentration is reached, symptom severity starts to decrease (11). It has been suggested that virus multiplication in the shoot may be controlled by two distinct root factors, the supply of cytokinins and the amount and nature of the nitrogenous compounds present (135). A tolerant cultivar Virocross contained more gibberellins, more cytokinins, and a lower concentration of amino compounds than a susceptible one (135). Because the sugars available for growth are less than in healthy plants, it has been recommended that infected ones be grown at somewhat lower temperatures, with free ventilation, adequate root moisture, and frequent water sprays for the foliage (67). There is no doubt that experienced growers can do much to alleviate the effects of infection, but it does not seem possible to greatly minimize them by altering nutrition. Early experiments on the effects of nutrients on symptoms showed that lack of potash but not of any other major nutrient increased the incidence of necrotic streak (1) and that the addition of lime or sulfate of potash retarded symptom development (131). On the other hand, excess potash in relation to growth increased susceptibility to infection. Attempts to mitigate the effects of virus infection by weekly foliar sprays with an aqueous solution of urea, with or without solutions of monoammonium phosphate and potassium sulfate, failed to affect fruit yield or quality sufficiently to warrant commercial use (36).
Fruit Quality and Yield

Tomato fruits can react to stresses in the plants in a limited number of ways, so it is not surprising that the quality defects that occur have been attributed to several causes. The yellow patches associated with the aucuba strain of TMV, and the severe blistering and necrosis caused by double virus streak were recognized, but for a long time there was considerable confusion over the very common unevenness of ripening referred to as blotchy ripening or severe mottle, graywall, cloud, internal browning or bronzing of the fruits.

The suggestion that internal browning or bronzing might be caused by TMV infection was made as early as 1941 (69) but it was 1956 before it was established that it can be induced as a shock reaction some three to four weeks after invasion by the common tomato strains of TMV, affecting only fruits that were already set when the virus became systemic (19, 22, 23, 28, 76, 140). TMV isolate, high soil moisture, low nitrogen and boron in the soil, and cultivar markedly affected the incidence and severity of this disease and of blotchy ripening (13, 21, 141). Internal browning is particularly evident in unripe fruit and usually is restricted to the fleshy parenchyma whose cells often collapse. It is most prominent near the shoulders of the fruit at the stem end, and most severe between the vascular bundles, which are not affected. White tissue as well as necrosis occurs frequently in the pericarp (85).

For a period there was considerable controversy over the causes of internal browning, some workers asserting that nutritional imbalance affected its incidence (127, 139); others thought its cause was environmental (62). The superficially similar graywall symptom was usually induced in the absence of TMV especially when plants were grown at low light intensity, low air temperature, and high soil moisture (100, 101, 136), but these noninfected fruits frequently have prominent necrotic streaks in the vascular region in the lower half extending to the stylar scar. However, the subject is again confused because some workers now attribute both graywall and internal browning to TMV infection (20, 43); it has been suggested that TMV and bacteria enhance the development of graywall, bacteria multiplying more in TMV-infected than in TMV-free fruits (138). Experiments under glass in the UK showed clearly that necrotic pitting, especially around the calyx, bronzing or internal browning, and severe mottle affect some but not all fruits that are already fairly mature when TMV invades them (28, 76). Many fruits with necrotic pitting fall off prematurely, and calices of affected fruits are dry and crinkled. The severe shock reaction lasts up to almost eight weeks after systemic invasion by the virus. The proportion of seriously affected fruits gradually decreases with successive harvests (28). The reason that most growers had not previously attributed these symptoms to TMV infection was that virus invading via the roots makes its way into the lower fruit trusses before penetrating into the young shoots; thus, fruit defects may be visible several days before leaves show symptoms. When leaf infection occurs, trusses show symptoms in a sequence that depends both on leaf position and the number of trusses already formed (40). While internal browning or bronzing, necrotic pitting, and severe mottle usually are caused by TMV infection, especially when the plants are lush in growth, one cannot always be sure of this; superficially similar
fruit defects also can be caused by adverse environments and nutritional imbalance in the absence of virus (79).

In addition to poor quality, fruit yield can be adversely affected by TMV in two ways—fewer fruits or smaller ones (28). Many estimates of loss have been made on both outdoor and indoor crops but most have been underestimates because the control plants became infected during the experiments. The experiments were then comparing early with late-infected plants, rather than infected and non-infected ones. Loss differed with cultivar, virus strain, growing conditions, and time of infection (5, 94). Some workers found that the earlier the plants were infected the greater was the loss (1, 18, 44), whereas others found that early inoculation decreased yields most in the early and midharvest pickings but in later pickings yields were less in late- than in early-infected plants (157). Experiments on the seasonal pattern of fruit production in tomato showed that infected plants always produced less than healthy ones but the magnitude of the loss varied with time of year and age of plant, being greatest in spring and in young plants (32). Losses have varied considerably in the different trials reported, but most fell within the range of 5 to 50%, and most losses have been due to fewer fruits rather than smaller ones (18, 130).

Experiments under glass in the UK, in which the control plants remained non-infected, showed similar losses with widely different dates of infection, from before any fruit had set to after it had all done so; with early infection there were fewer fruits, with late infection smaller ones (28, 29). Trusses coming into flower or setting at the time the virus became systemic, suffered the most in terms of amount of fruit set (28). Desiccation affected both stigmas (which were less sticky) and pollen (which germinated poorly) for about six weeks after inoculation. Plants inoculated in the seedling stage produced better pollen than plants inoculated later (120).

The factors affecting crop losses are so numerous and varied that it is impossible to more than guess at an average figure: somewhere between 15 and 25% loss in yield is probably about right. On a worldwide scale this forms a tremendous loss, whether in fruit tonnage, wasted manpower, or monetary value. In addition to losses in yield, many growers have suffered financially because badly blemished fruits were unsalable early in the season when prices are high. Before quality defects were recognized as a result of TMV infection, growers maintained that the greatest financial losses are incurred when TMV affects a crop before the fourth truss is flowering and that later infection was of little consequence. However, if quality defects make much of the early fruit unsalable, the total financial loss is often greater when infection occurs later (28). These considerations led to the development of deliberate early inoculation (see below).

**SOURCES AND TRANSMISSION OF VIRUS**

**The Tomato Crop**

**SEEDS** There was controversy for many years between growers, who maintained that TMV was seed-borne in tomato, and plant pathologists who said it was not,
because they were unable to obtain any infected plants by sowing seeds from infected fruits and growing the seedlings in isolation (25, 80, 130). Early workers who claimed that virus was in the embryo used relatively few seeds with inadequate controls; however, when 2000 tomato seeds were dissected in Australia, virus was found in the testa but not in the endosperm or embryo (45). Other workers also found some testae infected but showed, in addition, that the endosperm sometimes carried TMV (30, 142). Infected seeds were sometimes necrotic, especially those from early-infected plants; such seeds tended to contain virus in the endosperm (30). Severely necrotic seeds often failed to germinate. About half the seeds from infected fruits carried TMV, the proportion differing with tomato cultivar, time of infection, truss position, and method of cleaning (30). Seeds usually carried the virus externally, depending on the method of cleaning, but about a quarter also carried it within the testa or the endosperm. High virus concentrations sometimes occurred internally when the endosperm was infected but no evidence of embryo infection has ever been obtained. Endosperm infection occurred mainly in fruits that set after the plants were systemically infected, and decreased with increasing age at the time of infection. Thus there would seem to be a critical period during which the endosperm can become infected.

TMV persists in or on the testae for several months or even years after harvest. At first this was thought to depend on the way in which the seeds were stored (103); but this was not substantiated when seeds were stored in open dishes or in closed glass tubes (30). In both, virus concentration remained high in stored seeds for nearly a year and then fell and remained at a constant low level for five years; presumably the virus on or in the testa (but not that in the endosperm) was gradually inactivated. Some seeds extracted from fruits by fermentation remained infected internally when stored in paper packets for nine years (30). Some TMV strains survived only a few days on germinating tomato seeds, and it was suggested that these contained substances that inhibited TMV, differing with tomato cultivar (125). However, other workers grew tomato embryos in media containing virus but found no evidence of inactivation (46, 153). Rapid inactivation of virus was observed in seeds after sowing, perhaps caused by soil microflora, but testae remained infective for at least ten days. This was long enough for contamination during transplanting (30, 150, 153).

It is clear that TMV is seed-borne in tomato, but because it does not occur in the embryo, is this a source of virus for the crop? It has been shown that seedlings are not infected when left undisturbed, but growers often transplant the young seedlings a few days after germination. Loosely held TMV on the surface of seeds may be the most likely source, contaminating the seedling and entering through mechanical abrasions made during transplanting; in such cases, most infections were presumed to occur after the testae were carried above ground on the cotyledons (142). However, other work indicated that infection usually occurred below the soil surface when testae or the remaining endosperm were pressed against the seedlings during transplanting (30). Normally very few seedlings were infected in this way because the testae often were left behind in the compost when the seedlings were transferred. Also much of the virus carried externally was inac-
activated after a week in the soil, and very young seedlings resisted infection. Seedlings were not infected by absorbing TMV from the endosperm during germination because none was infected unless transplanted.

Only a few seedlings need to be infected in a tomato crop for the virus to spread rapidly. A weekly survey of 374,000 seedlings from thirteen different sources of seed on ten commercial holdings found 0.05% of them infected and although these were removed, virus had been spread to other young plants that formed sources of TMV for 7 of 22 growing houses to which the seedlings were transplanted (34). Direct seeding has been adopted by some growers to eliminate prickling out; this method is easier now that it is possible to obtain pelleted seeds.

**SHOOTS AND ROOTS**

Other plant parts that can act as virus sources are the leaves and shoots, and the roots, or the remains of both. Handling the plants while tying them, removing side shoots, or picking fruits is undoubtedly the most important method of spreading TMV within tomato crops (34). Many growers attempt to delay virus spread by handling last all obviously infected plants; but this is seldom effective because virus is in the leaves some days before they show symptoms. The longevity of the virus in dried plant debris poses a special problem for tomato growers. Plant debris is usually cleared up from the soil surface between crops, but small scraps of infected leaves may adhere to parts of the glasshouse structure. Root debris left in the soil is a different matter and one that was long overlooked by growers. This is surprising because in 1928 it was shown that TMV remained active for at least 70 days in glasshouse soils that had carried infected crops, and tomato plants were infected when planted into such soils. Soil conditions affect virus survival, TMV remaining active longer in heavy than in light soils (78). Powdered leaf debris lost infectivity within a month in moist soil but remained infective for two years in dry; also, root debris remained infective for six months even in moist soil. Tomatoes were infected when planted into glasshouse soils within five weeks of clearing infected plants but not when a different crop was grown for six months in between the tomato crops (78, 80).

Root debris from previous crops is undoubtedly the most important source of virus for tomatoes under glass (29, 53). Earlier workers were correct in stating that the proportion of plants that became infected in this way is usually small (but see 137) but later spread within the crop is so rapid that only a few foci are needed to start an epidemic (53). It makes no difference if the plants are set out into soil containing root debris or into a layer of sterilized soil covering it; once the roots penetrate into contaminated soil they may be infected (29). The virus was shown to persist in tomato root debris at a depth of at least 120 cm for at least 22 months in fallow soil, and for over two years in soil under black polyethylene that carried a layer of clean compost above it (35). Transmission presumably occurs when root hairs are damaged in the presence of infected debris or free virus leached from it into the soil (29, 128). TMV has even been detected in surface water used for watering the tomato crop in the Netherlands (148). There is no evidence to suggest transmission to neighboring plants via root contact or anastamosis (29), although it may occasionally occur from virus exuded into the rhizosphere from infected plants (98).
INSECT AND BIRD TRANSMISSION Some early work on insect transmission of TMV is suspect because of inadequate controls and the ease with which the virus is transmitted by contact (87, 105). However, recent work has shown that glandular leaf hairs are susceptible to injury by clawing. Aphids can pick up TMV from tomato hairs and subsequently inoculate healthy plants by clawing them (24, 64). By feeding aphids (*Myzus persicae*) through a membrane on a solution containing TMV to minimize the chances of their claws being contaminated with virus, it was shown that they could acquire TMV only when it had been mixed with poly-L-ornithine or poly-L-lysine, although the reasons for this are not clear (116, 117).

TMV-like particles were found in the guts of some sucking insects fed on infected plants (107) and in aphid honeydew (119), but TMV could not be detected in droplets emerging from the cut stylets of *M. persicae* which had been feeding on infected plants (149). If insect transmission of TMV occurs in tomato crops it must be of minor importance relative to other methods.

It seems obvious that a virus so readily spread by contact might be carried within crops, and from one crop to another, by birds on their feet, plumage, or beaks, or by mammals on their fur. Wild animals occur in outdoor crops and occasionally birds enter glasshouses, fly among the plants, and even peck the fruits. These can undoubtedly transmit TMV within tomato crops (31).

Alternative Host Plants

WEEDS Viruses of the TMV group occur in a wide range of cultivated plants and weeds, but it has seldom been determined if these are sources of virus for tomato crops. TMV is commonly found in *Plantago* (70) but it differs from the common tomato and tobacco strains (65). TMV overwintered in rootstocks of *Physalis subglabrata* in Indiana (60), and field investigations suggested that this weed was a source of virus for outdoor crops. Apart from this there is little evidence that weeds play much part in the epidemiology of tomato mosaic.

SMOKING TOBACCO The relative importance of smoking tobacco as a source of TMV in tomato crops used to be as controversial a subject as seed transmission. It is only during the last fifteen years that it has been realized that the virus strains common in tomato are different from those in tobacco. It is possible that the strain prevalent in tomato in the 1920s was the tobacco form which later mutated after frequent passage through tomato; but the latest research does not support this view (124).

Many workers found most cigarettes, cigars, pipe and chewing tobaccos infected (3, 26). The habit of chewing tobacco was incriminated as a common cause of infection for many tobacco and tomato crops, because of the infective saliva on the hands. Pruning knives, also, were often kept in pockets containing tobacco dust (71). There is no doubt that tomato can be infected with the tobacco form (3, 26). The speculation was removed from the subject when forms of White Burley tobacco were selected, which reacted to inoculation with most tomato strains of TMV with necrotic local lesions only, whereas the common tobacco strains
caused systemic mosaic and no local lesions (97, 143). These and other test plants made it possible to survey tomato crops for various strains of TMV. As a result, it is now known that the tobacco strains of TMV can and occasionally do infect tomato, but in general tobacco is not an important source of virus for the tomato crop (17, 26, 55, 82, 83, 124, 151).

Clothing

Although some workers had shown that TMV remained active on clothing and could be transmitted also by machinery passing through crops, the importance of contaminated clothing was long overlooked by growers and plant pathologists. A survey of clothing worn by workers in a major English tomato growing area in 1962 indicated that 42% of outer garments had not been cleaned during the year; 24% of them had been worn the previous year and two thirds of these had not been cleaned between seasons (33). That infected clothing is a potent source of virus was shown by a worker wearing a cotton overall for two days while working in an infected crop and then walking between rows of healthy plants for 10 min so that the overall brushed the leaves: 19 of the 20 plants were infected but none of the controls.

Washing the infected clothes with detergents in hot water, or dry cleaning them, decreased the TMV content. Virus on clothing stored in sunlight was inactivated within a few months. TMV-impregnated clothing stored in a shady place remained infective throughout a winter, and when stored in the dark remained so for over three years. There is no doubt that nursery workers, extension advisers, and firms' representatives can easily carry virus from one crop to another as well as spread it within the crop. However, it is doubtful if clothing forms a major reservoir of overwintering virus.

Structures

It used to be assumed that TMV could survive between crops on glasshouse structures but tests showed that infective sap was rapidly inactivated in daylight, occasionally persisting at low concentrations for about two months (33). A survey confirmed that TMV seldom overwintered on commercial glasshouse structures although it might persist in small fragments of dry tomato leaves if the houses were not washed. The wires used to support crops were occasionally still contaminated after four months in storage, but could be cleaned by heat or chemical inactivators (103).

VIRUS MOVEMENT IN TOMATO

The development of disease in the tomato plant depends not only on the virus strain, cultivar, and cultural conditions but also on movement of the virus once it has effected entry. This in turn is influenced by the site of infection, and a knowledge of this movement is important in understanding the epidemiology of the disease. The development of symptoms in the young leaves around the growing points following leaf inoculation depends upon the age of the plant and time
of year, young plants often showing symptoms in five days in summer but taking three weeks in winter, and taking several days longer in older plants than in seedlings (2, 9). When infection occurs through a leaf, the virus multiplies therein for a few days and then moves into the phloem; from there it is carried rapidly first to the roots and then to the shoot apices, or to both simultaneously, bypassing many parts of the plant en route (37, 129). Large plants may never become completely invaded. Because the tomato plant grows by a succession of axillary meristems, which terminate as fruit trusses, lower trusses often are infected before virus passes to the growing point (133) and so may show severe fruit blemishes before leaves show symptoms.

When plants were infected via the roots, the virus often failed to invade the tops especially when growth was slow (59, 128), although young seedlings inoculated in the roots usually became systemically infected (122). The virus sometimes is localized in the root around the initial entry point. Systemic invasion can vary with individual plants and with the time of year; it is common in summer but rare in winter (128). Experiments to test the frequency of root infection and the time taken for virus to become systemic showed that TMV could be detected in young shoots before symptoms appeared. The periods between inoculation of roots and symptom development in the shoots vary from three weeks in young plants to six months in older plants (the average periods being between ten and sixteen weeks). Such periods are shorter in summer than in spring or winter (29). In some older plants the virus did not leave the roots during the experimental period of about six months. Seedlings showed leaf symptoms an average of five weeks after inoculation in the roots, although some did not do so in ten weeks. Symptoms appearing soon after planting out could well result from infection of the roots during transplanting (122). The lower fruit trusses often showed severe blemishes before the foliage showed mosaic, being invaded by the virus before the upper shoots. This undoubtedly contributed to the failure of growers to attribute such fruit symptoms to TMV.

The frequency of infection by root contact with debris from the previous crop was low, but a few primary infections can lead quickly to a serious epidemic of the disease. The sudden development of symptoms in previously healthy commercial crops in the UK during May and June probably results from spread by handling during tying and sideshooting, following a few initial infections through the roots.

CONTROL

Prevention of Infection

Knowledge of the epidemiology of the disease now makes it much easier than before to grow a healthy crop, but it usually is impossible to prevent infection under commercial growing conditions.

CLEAN SEEDS OR DIRECT SEEDING Seeds taken from healthy plants can be sown, and the seedlings planted into clean soil but it is essential to test the mother plants
and the seed batches by inoculation into *Nicotiana glutinosa* or some other test plant to ensure that fruits are not harvested from late-infected plants. In the Netherlands, some growers use an electronic scanning device to sort out necrotic seeds, because these germinate poorly and are often infected in the endosperm (30, 124).

If infected seeds have to be used, infection of the crop can be prevented by direct sowing into the final positions, so that the seedlings are not transplanted (30, 52, 61). Recent cultural changes, such as seed pelleting and the development of plastic containers and compost blocks, have made direct seeding a commercially viable practice.

**ISOLATION** The crop should be kept isolated from sources of infection. This means planting outdoor crops into soil that has not carried tomatoes, tobacco, or other plants susceptible to TMV for one or preferably two seasons. If this cannot be achieved under glass, the contaminated soil can be isolated with sheets of polyethylene and the plants grown in a suitable compost above this (159). This method was used by several UK growers for a time but has been superseded by the bag method—isolated plastic bags, or troughs being filled with peat compost (156). This is now the predominant method of culture in Guernsey and in a few other areas of the UK.

The latest method to isolate the roots from contaminated material is the waterbed method (41), a British hydroponic technique in which the tomato roots are immersed in a flowing stream of water containing dissolved nutrients in lay-flat plastic tubing. If clean seeds are used, the crop ought to be kept free from TMV, but in view of the Dutch observation of TMV in irrigation water (148), if a plant did become infected by contact with a worker's hands or clothing there would be the danger of rapid spread throughout the house via the water. Another technique that proved successful is to grow the plants on straw bales isolated from the soil by polyethylene and watered with nutrient solutions, but this did not become popular. Isolation by distance from infected crops is also desirable to minimize the transmission of virus by birds, mammals, or insects.

**HYGIENE** The following measures are desirable but it is unrealistic to expect them to be practiced by commercial growers and their employees. All tomato crop debris should be disposed of at a sufficient distance from growing crops to prevent it becoming a source of virus. Workers should not be asked to tend infected as well as healthy crops, and should not wear uncleaned outer clothing that had been worn in infected crops. If these measures are not possible, it is little use working symptomless areas first, which is a common practice. Visits by managers, foremen, advisory officers, or commercial representatives should be minimized. All persons entering a healthy crop should be obliged to put on clean overalls and to wash their hands; visits should be restricted to one pathway in a crop. Workers should be made aware that TMV can be carried on their footwear and hair as well as on clothing and hands.

Although smoking tobacco is only a minor source of infection, smoking should be forbidden in tomato crops and smokers should wash their hands before tend-
ing the crop. They should not keep tools in pockets that might contain tobacco debris.

**Virus Inactivation**

**IN PLANTS AND SEEDS** Attempts to inactivate TMV in infected plants or decrease the effects of disease have not proved practicable (14, 88, 160), although β-3-indolylpropionic acid or α-naphthalene sprayed on field-grown tomatoes in India were reported to alleviate symptoms (16). Much more work has been done with infection inhibitors or virus inactivators on the surface of plants, particularly cow's milk. Following an initial report that spraying tomato foliage every ten days with milk, diluted with water, eliminated TMV spread (102), numerous tests have shown that skimmed milk sprayed on to the plants, and used to wet the worker's hands shortly before tending them, have prevented or delayed TMV spread (63). However, there are several reports of poor results from such treatments (47, 66); they delayed but did not prevent the spread of TMV in the crop. Such treatments would not be worthwhile where late infection causes internal browning or other fruit defects; but they have been shown to increase significantly yields of field crops (51, 75). The casein in milk is the strong inhibitor, lactalbumin and α-lactoglobulin being weak ones (74).

If possible, tomato seeds should be harvested only from plants shown by testing to be virus-free. Such plants are seldom available, and if they are not, seeds should be harvested from the lower trusses of late-infected plants so that there is a good chance that they will be free from internal virus. In the past, most commercial seed stocks have been infected. Several attempts have been made to inactivate virus on or in seeds by chemical means. After extraction from the fruit, most tomato seeds are cleaned by fermentation or by a chemical treatment such as adding sodium carbonate solution or concentrated hydrochloric acid to the pulp. They are then washed in water and dried. Many investigators claim that these treatments, especially the HCl, free contaminated seeds from TMV (6, 39, 58, 104) but others, who probably worked with seeds that contained virus internally, were unable to free all batches of seeds completely (30, 47, 72). Similarly, treatments of seeds with trisodium orthophosphate or sodium hydroxide, or with ultraviolet light have sometimes been successful, sometimes not (6, 30, 103).

Heat treatment (three days at 70°C or one day at 80°C) of dried seeds has also been used successfully; it is the only method that is successful for seeds infected internally, although three weeks at 70°C did not free those infected in the endosperm (30, 73, 84, 155). Heat treatments may delay germination but no deleterious effects occur if seeds that are newly extracted are adequately dried first; such seeds will withstand at least 70 days at 70°C (126). Heat-treated seeds are now readily available commercially.

**TMV INACTIVATION IN DEBRIS** TMV in soil and in plant debris can be destroyed by heat. Virus in undiluted plant sap is inactivated in 10 min at 93°C or in 80 min at 90°C (118); but in dried debris it may survive higher temperatures (12, 35).
The length of exposure necessary at different temperatures depends on the concentrations of virus and its location—whether in sap or in leaf or root debris (35).

Steaming of glasshouse soils, as done commercially, may not eradicate TMV even from the upper soil layer because an adequate temperature is not maintained long enough to penetrate thick roots or to reach every part of the soil, especially that near brickwork, water pipes, or other structures (53, 154). It is extremely difficult to inactivate TMV in thick roots by steaming the soil; as many large roots as possible should be removed before steaming (53). Steam penetration is best in dry soil but dry roots are more difficult to free from TMV than moist ones. Sheet steaming gives satisfactory results, but the effectiveness of all methods depends upon the structure, texture, and moisture of the soil. Even if all the TMV in the upper 30 cm or so of soil is inactivated, however, debris at lower depths can remain infective for several months, particularly in fallow soil (35).

Although formaldehyde inactivated purified TMV in laboratory tests (81) and considerably decreased virus concentration in debris (35), treatment of debris-containing soil with formalin did not prevent infection of tomato seedlings (86).

When concentrations of five times the recommended doses of chloropicrin, metham sodium, methyl isothiocyanate, D-D, and nabam were tested, these soil fumigants did not inactivate TMV in plant debris or in soil or when they were mixed with infective leaf sap. Indeed, the main effect of treating buried roots with chloropicrin and metham sodium was to increase the proportion of root fragments that remained infective over a period up to 13 months after treatment, from 33% in the untreated controls to 61% and 73% respectively (35, 77, 89). The consequence of such chemical sterilization was an earlier attack of TMV in the crop through root infections. There was no clear indication of a causal relation between the persistence of TMV and the microorganisms colonizing roots although the population of fungi and bacteria, many of them involved in the breakdown of vegetation, were significantly affected by the treatments (35). Only methyl bromide destroyed all the TMV in root fragments in closed containers but it might not be effective under glasshouse conditions because, although it inactivated TMV in thin roots, it did not do so completely in thick ones (77, 108, 147).

**TMV Inactivation on Hands, Tools, and Structures**

It is not easy to eliminate TMV from the hands after working in an infected crop, and just washing them with soap and water is inadequate. One of the best methods is to wash in a 3% solution of trisodium orthophosphate and then scrub well with soap and water, although even this does not always eliminate virus in the areas under finger nails (27, 99).

Tools can be heat sterilized, or dipped in 3% trisodium orthophosphate (27, 99, 103). Detergents and washing-soap solutions only inactivate virus on tools, wires, etc if these are soaked for long periods in high concentrations of the solutions. Although light soon inactivates TMV adhering to glasshouse structures, small fragments of leaf debris may carry the virus, and so thorough washing of the houses with a disinfectant, when they are empty between crops, is to be recommended.
Minimizing Fruit Losses

ALTERING THE ENVIRONMENT  Although growing conditions affect the plant's response to virus infection, there is no concensus as to the optimal environment to minimize loss. Many reports are concerned with leaf symptom severity, whereas all that matters is salable fruit yield. Because the sugars available for growth in infected plants are less abundant than in healthy ones, it has been suggested that infected plants under glass should be grown at less than 27°C by day and a few degrees lower than healthy plants by night (67). Free ventilation, frequent water sprays, and adequate root moisture also have been recommended to aid the growth of infected plants in sunny weather.

Some workers have suggested that such symptoms as internal browning and blotchy ripening may be enhanced by heavy watering, or inadequate nitrogen and boron (134, 139); others did not find the incidence of internal browning affected by nitrogen, although blotchy ripening was enhanced by high nitrogen (42). These severe fruit reactions to virus infection are much worse when vegetative growth is abundant and when watering is excessive. When growth is better balanced, leading to a high dry matter ratio, they occur less frequently or not at all (10).

DELIBERATE INOCULATION WITH TMV  The discovery that late infection with TMV often causes severe fruit blemishes which are not present when the plants become infected before any fruit has set, led to experiments which confirmed that deliberate early infection would be worthwhile for a grower who always suffered quality loss (28). Plants infected before the first truss set often suffered less weight loss than plants infected later. Thus, on the average, the financial returns were greater from inoculated crops than from noninfected ones that suffered late infection and defects in fruit quality.

Growers in the Isle of Wight (UK) were the first to adopt deliberate inoculation in 1964, although a mild strain of TMV was not available and the normal, fairly severe strain of tomato mosaic was used for inoculation in the seedling stage. The proportion of unsalable and poor quality fruit was reduced from about 30% to under 3% in 1965. The benefit to be gained from seedling infection would be greater if a mild strain of tomato TMV could be used that would protect the plants against severe strains, and attenuated strains that did this were obtained by prolonged heat treatment (106, 109, 110). Then in 1968 an almost symptomless mutant (MII-16) which gave good cross-protection was isolated in the Netherlands by the mutagenic action of nitrous acid (121, 124).

Tests on commercial crops proved very satisfactory in decreasing crop loss, and a spray gun method of inoculation was found to be better than manual inoculation because of the risk of contamination with other strains (121). Such inoculation with the mild strain also prevented the development of severe strains acquired through seedling root contact with inoculum (123). However, it may not give complete protection against all naturally occurring strains of TMV, either of the tomato or tobacco forms (57, 96).
The mild strain, when inoculated into seedlings, caused a temporary inhibition of growth and delayed flowering and fruit set, so it was necessary to advance the sowing date by about a week to compensate for this (124). Seeding inoculation with this strain resulted in negligible internal infection of seeds in subsequent fruits, in contrast to plants inoculated with the parent strain when endosperm infection occurred, so deliberate inoculation with the mild strain could be used by seed growers together with subsequent heat treatment, when seed plants cannot be kept healthy.

The mild strain, MII-16, is now widely used by commercial growers in the UK (57) and other countries, as well as in the Netherlands, and apart from the breeding of resistant cultivars, its development has done more than any other factor to decrease crop losses from TMV in tomato. Largely as a result of such inoculation, average yields of early heated crops in the Netherlands increased by 15% between 1971 and 1973, and trials in the UK in 1971 gave increased yields of 7% (146). The very success of the method may well make growers reluctant to accept resistant cultivars unless these can be shown to have considerable advantages over existing ones (124).

Cross-protection between virus strains is dependent on temperature; a mild strain that prevented the multiplication of a severe one at 15 or 20°C did not do so at 25 or 30°C (145), probably because high temperature has a detrimental effect on the multiplication of mild strains (112). The mild strain MII-16 was inactivated when stored in dried leaves for a long period, and it was found necessary to freeze concentrated suspensions for commercial use (124).

Most of the work on inoculation of seedlings with attenuated strains of TMV has been commercially successful, but phenotypic mixing of strain characteristics can occur with TMV strains, producing new, recombinant strain types. M. Hollings has warned (in litt.) that if MII-16 is widely used over a lengthy period, it may saturate the soil in tomato houses and may combine with more virulent strains. Because MII-16 is derived from a strain 1 type, any new recombinants will stand a good chance of breaking resistance in Tm-l plants. Also the attenuated strain can increase considerably in virulence during passage through some test plants. Further, MII-16 multiplies much more slowly than typical TMV strains in tomato, thus offering a poor chance of this strain surviving in competition with more virulent strains against which it does not protect.

Breeding Resistant or Tolerant Cultivars

An adequate review of such a large subject as tomato breeding for disease resistance would be out of place in one on epidemiology, and the subject has been well reviewed elsewhere (11]). Cultivars is now the most widely practiced means of preventing losses from TMV infection in several countries, a brief account of recent work is essential.

The success achieved so far suggests that the development of commercially acceptable resistant cultivars is not only possible but also may result in long-lived resistant or immune cultivars, despite the apparent ease with which TMV can
mutate. The several strains of TMV that occur in different parts of the world are not identical and this complicates the problem (7, 95). Field resistance was sought without much success, so the breeders have relied on three major gene factors for resistance, Tm-1, Tm-2, and Tm-2'. Five strains of TMV have been identified in tomato in Europe: strain 0, the commonest, induces no symptoms on plants with any of the resistance factors; strain 1 causes symptoms on Tm-1 plants, strain 2 on Tm-2 plants; strain 1:2 causes symptoms on both and also on Tm-1 Tm-2 plants. Strain 2' induces symptoms on Tm-2' plants, but it is rarely found. Only strains 0 and 1 are common (113, 114, 125). This work is based on the resistance that exists in wild Lycopersicon species, e.g. L. peruvianum. The mechanisms of resistance are not fully understood, but virus multiplies very slowly in resistant plants and concentration remains low in contrast to susceptible plants (7, 49, 115).

There are differences of opinion on the plant breeding policy to be followed, some breeders thinking it worthwhile to release cultivars with single gene resistance even if they last only for limited periods (8). In the UK, tomato crops sampled prior to the introduction of cultivars with monogenic resistance to TMV yielded only strain 0, but within two years of the introduction of cultivars heterozygous for Tm-1, strain 1 was found with increasing frequency (114). Strain 1 continued to increase following the introduction of cross-protection with the avirulent TMV strain MII-16 (originally derived from a strain 1 isolate) and two years later 94% of isolates sampled from nurseries using the technique were of strain 1 in contrast to 39% from other nurseries (54). It was suggested that this increase in the incidence of strain 1 increases the risk of breakdown of resistant cultivars and that it is desirable to achieve multigene resistance, preferably with all the three known factors, in a cultivar before it is released into commercial production (114, 144).

Unfortunately, when some of the resistant cultivars are grafted on to TMV-susceptible rootstocks, the virus invades the shoots and causes severe fruit necrosis and fruit drop. Recently a rootstock has been introduced with single-gene resistance (Tm-2') but again there is the danger of selection of new TMV strains if a three-gene scion is grafted on to a one-gene stock; attempts are now being made to produce a rootstock carrying Tm-1, Tm-2, and Tm-2' (48).

CONCLUSIONS

Almost all aspects of the epidemiology of tomato mosaic have been reexamined during the last twenty years: The work is being done in several countries, which illustrates the importance attached to the disease. Many myths resulting from inadequate early research have been dispelled, and the sources of virus and its transmission are now well understood.

Infected seeds and root debris in the soil are the two most important sources of TMV. The virus is easily carried from one crop to another on clothing as well as on hands and tools. Hygiene measures necessary to prevent TMV introduction and spread are too uncertain and involve complications which make them com-
mercially unacceptable, so the development of a mild-strain inoculation technique that minimizes fruit losses and protects the plants against invasion by more damaging strains has been of great importance. However, virus epidemiologists have never been very happy about deliberately disseminating viruses, because of the risks of selection of virulent strains, and would much prefer to try to eliminate the virus. Breeding for resistance to TMV is going ahead in several countries and so far several resistant cultivars have been accepted in commerce. It remains to be seen if the plant breeders can keep ahead of the selection pressures on the viruses. If they can, the great reservoir of tomato strains of TMV should gradually be reduced, and healthy crops become the norm.

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