A. Th. B. Rast

Institute of Phytopathological Research, Wageningen, the Netherlands

Variability of tobacco mosaic virus in relation to control of tomato mosaic in glasshouse tomato crops by resistance breeding and cross protection

Centre for Agricultural Publishing and Documentation

Wageningen – 1975

ISBN 90 220 0559 3

The author graduated on 6 June 1975 as Doctor in de Landbouwwetenschappen at the Agricultural University, Wageningen, the Netherlands, on a thesis with the same title and contents.

This report will also be published as Mededeling No. 689 of the Institute of Phytopathological Research (IPO), Wageningen and Publikatie No. 192 of Proefstation voor de Groenten- en Fruitteelt onder Glas, Naaldwijk.

Abstract

RAST, A. TH. B. (1975) Variability of tobacco mosaic virus in relation to control of tomato mosaic in glasshouse tomato crops by resistance breeding and cross protection. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 834, ISBN 90 220 0559 3, (viii) + 76 p.,15 figs, 22 tables, 104 refs, appendix, Eng. and Dutch summaries.
Also: Doctoral thesis, Wageningen., Publties Proefstn Groenten- en Fruitteelt Glas Naaldwijk 192 and Meded. Inst. Phytopath. Res., Wageningen 689.

Various strains of TMV occur in susceptible crops of tomato. This variability is of importance to resistance breeding and to cross protection as alternative methods of control. Strains of TMV differ in the kind of symptoms expressed and in the extent of the host range. Symptom variation allows for the distinction of eight strains of which the tomato strain is the most important. On a host range including *Solanum pennellii*, *Lycopersicon esculentum* CStMW-18 and *L. peruvianum* four strains are differentiated which correspond with Pelham's Strains 0, 1, 2 and 2^a. Strains of TMV also differ as to longevity in vitro. The prevailing Strain 0 and Strain 1 are more persistent than Strain 2. The instability shown by Strain 2^a during isolation suggests that in practice it may not easily overcome the resistance of the gene Tm-2^a. The symptomless nitrous acid mutant MII-16 which is discussed in relation to cross protection is also less persistent than its parent tomato strain. This necessitated a careful preparation of the inocula for commercial use.

Contents

1 Introduction

Tomato mosaic caused by strains of tobacco mosaic virus (TMV) is the most widespread virus disease of tomatoes and has probably been a problem ever since the crop was grown in glasshouses. The root of the problem lies in the very nature of the causal virus which is the most infectious and persistent plant virus. In addition TMV may be reckoned among the most variable viruses as evident from the existence of numerous strains. This variability has a different significance when related to the different approaches of controlling the virus.

When control is intended to prevent infection at all costs the knowledge of differences between strains is of little practical importance. Therefore in extensive studies on the epidemiology of tomato mosaic interest has been confined to the prevailing strains associated with the disease while those causing deviating symptoms received only casual attention (Broadbent, 1961).

For resistance breeding, from which the ultimate control of tomato mosaic is expected, the variability of TMV is of vital importance. Strains, not distinct by their symptoms, have been discovered to show differences in pathogenicity on certain resistant breeding lines of tomato (McRitchie & Alexander, 1957). Furthermore strains have been observed to adapt and overcome the resistance under investigation (Pelham, 1972).

For cross protection as a method of control in which tomato seedlings are deliberately inoculated with a relatively harmless strain to protect them against infection by more severe strains, the knowledge of strain interrelationships is of particular interest. While natural strains may be used for the purpose (Fletcher, 1968) further improvements have been obtained with heat-attenuated strains (Komochi et al., 1966; Paludan, 1968) or artificially induced mutants (Rast, 1972). So, the same variability which represents a threat in resistance breeding may be exploited in cross protection.

It should be realized that the variability of TMV inevitably results in the occurrence of mixtures of strains which interact with the host and its environment. Further that in the competition between strains properties other than those governing symptom expression or pathogenicity may play an important role. These factors must be taken into consideration when studying TMV strains. The single-lesion method of isolation for example has proven its value for the separation of strains which differ in their symptom expression on one host, but requires the use of additional species for distinguishing strains different in host range. The customary method of storing TMV in infected dried leaves may be detrimental to strains kept for certain symptom characteristics or further pathogenic properties.

This thesis is primarily intended to give an account of ten years of work on the problem of tomato mosaic in glasshouse tomatoes in the Netherlands. Variability of TMV is considered in connection with three different methods of control viz. control by preventive measures, by resistance breeding and by cross protection. The first part (Chapters 2 and 3) deals with inadequate control by preventive measures and the variation of symptoms resulting from chance infections. The second part (Chapter 4) deals with pathogenic variation in relation to resistance breeding and the third (Chapter 5) with the use of a symptomless mutant in cross protection. In approaching the problem of tomato mosaic it was realized that little progress could be expected from preventive control measures. The reasons why no further attempts were made to find a solution in this direction have been outlined in Chapter 2. Efforts were concentrated on the search for suitable strains for testing purposes in resistance breeding and for cross protection. Initially an inventory was made of the symptomatology of different strains and these are described and classified in Chapter 3. Subsequent attempts to classify such strains on the basis of their pathogenicity were only partially successful because this factor may also be subject to variation as shown in Chapter 4. Much of this pathogenic variation is probably due to the fore mentioned inadequacy of the single-lesion method. This is suggested by the swift adaptive changes observed with strains of TMV following passage through certain hosts.

2 Prospects of preventive control of TMV in tomatoes in the Netherlands; a review of literature

2.1 The primary sources of infection

The ultimate control of tomato mosaic in susceptible tomato crops depends largely on whether or not the primary sources of infection can be completely eliminated. Seeds and root debris in the soil are generally accepted as the most important sources of TMV. Compared with these other sources like smoking tobacco, weeds, etc., play only a minor role in the carry-over of the virus (Broadbent, 1961). In the vast greenhouse region in the province of South-Holland, comprising the Westland district and the adjacent belt called De Kring, surface water is an additional source of TMV (Van Dorst, 1970).

2.2 Seed transmission and seed treatments

Transmission of the mosaic disease of tomatoes by tomato seed was observed by Westerdijk (1910) who concluded that the virus could infect embryonal tissue and that the disease was inheritable. Allard (1916), however found no evidence for transmission with transplanted seedlings and so excluded the possibility of embryo infection. These early reports are in a way characteristic for nearly half a century of controversies on the issue which remained unsettled until Broadbent (1961) published his review of literature. He not only gave a clear insight into the whole TMV-problem but by critically examining the experimental results obtained so far pointed out the gaps in knowledge to be filled by future investigations. Simultaneously Taylor et al. (1961) presented a review of seed transmission. These authors and Broadbent (1965b) established that TMV not only contaminated the outside of seeds but could also be detected within the testa and the endosperm. Its presence in the embryo, however, could not be confirmed with certainty. Furthermore Broadbent found that the proportion of infected seeds obtained from infected plants varied with tomato cultivar, time of infection and stage of development of the fruit at the time of infection. He also found that even the seeds of separately harvested fruits were not uniformly infected. Consequently large variations in contamination or infection may be expected to occur with any two batches of tomato seeds. This particularly applies to commercial seed samples in which Van Winckel (1965) found 0 to 94% of the seeds to carry TMV

In spite of the high rate of seed-borne TMV that is sometimes found when seeds are assayed, TMV is actually transmitted to a very limited number of seedlings. Since

the work of Broadbent (1965b) and others it is evident that transmission takes place only when seedlings are pricked out. Furthermore the chances for transmission are less with freshly emerged seedlings than with older ones (Broadbent, 1965b). Pricking out seedlings may bring them into contact with contaminated seed coats which may then cause infection. Studies by Taylor et al. (1961) and Van Winckel (1965), however, suggest that any contact during pricking out may suffice to cause infections, as they found both cotyledons and roots of seedlings to be contaminated by TMV. Roots appeared to be more often contaminated than cotyledons which in turn suggest that infection through the root could occur more frequently than infection of aerial parts. Although the possibility that seedlings become infected by the roots should not be ignored (Van Winckel, 1965) the chances seem rather small. Even when highly infective leaf sap is used the inoculation of roots will not necessarily cause infection. When it does, infection will usually take more time to cause visible disease symptoms than an above ground infection. Broadbent (1965b) found that seedlings became systemically infected within five weeks following root inoculation in winter. Results of small-scale experiments by Rast (1973) while confirming most of Broadbent's findings indicated that root infections occasionally require more time to develop into fully systemic infections. It is therefore conceivable that under commercial conditions most root infections, because of the low concentrations of TMV involved with seed transmission, remain unnoticed until after planting time.

The possibility of internal infection of tomato seeds and the irregularities in the distribution of TMV among batches of seeds probably explains a great deal of the controversial results obtained by former workers. Knowledge of these facts has resulted in a gradual change in the methods adopted for treatment of seeds. Whereas much of the earlier work dealt with the inactivation of TMV by chemicals, recent workers have investigated the effects of dry heat (Pécaut & Laterrot, 1963). Since Broadbent's extensive investigations it is taken for granted that chemicals like hydrochloric acid and trisodium phosphate are effective only against the virus on the outside of seeds. For the inactivation of internal virus heat treatment appears to be the only remedy. Howles (1961) reduced virus content of seeds considerably by submitting them to a treatment of 72°C for 22 days. Except for a delay in germination by two days no adverse effects were observed on the seedlings. His findings were confirmed by Broadbent (1965b), Laterrot & Pécaut (1965) and Rees (1970), who worked at 70°C. Equally favorable results were obtained with treatments of two and five days respectively (Broadbent, 1965b; Laterrot & Pécaut, 1965). Rees (1970) convincingly proved that seeds can withstand storage at 70°C even for months without losing viability provided seed moisture can escape freely during treatment. The use of higher temperatures increases the risk of poor germination and deformation of seedlings as shown by Laterrot & Pécaut (1965). They reported that exposure of seeds to 80°C for 24 hours, while drastically reducing virus content, only delayed germination but had no further effects on the seedlings. This treatment, considered unsatisfactory by Van Winckel (1967), represents about the upper safe limit beyond which damage is bound to

occur. Exposures to 85°C for 24 hours or 80°C for 48 hours causes some seedling deformation.

The results obtained with heat treatments in general are influenced by the location of the virus (Broadbent, 1965b), tomato cultivar (Pécaut & Laterrot, 1963) and such factors like age and moisture content of seeds (Rees, 1970). None of the treatments which so far have been tried were effective in completely eliminating TMV from infected seeds and so preventing its transmission. The use of treated seeds, however, obviously lowers the incidence of seed transmission (Laterrot & Pécaut, 1968).

2.3 Soil transmission and soil sterilization

After the removal of an infected tomato crop plant debris remains on or in the soil which then serves as a source of inoculum for the next crop. Broadbent (1961) makes a distinction between infection of stem and leaves occurring at or above the surface of the soil and infection of roots in the soil by contact with living infected roots, root debris or TMV released from them. There are many observations including those by Van Koot (1939) indicating infection of plants through their roots. These have been confirmed by careful studies of plants grown in TMV-containing media and on the consequences of root inoculations (Broadbent, 1965a; Roberts, 1950; Fulton, 1941). Plants allowed to grow in a substrate to which infected debris or infective sap had been added were eventually found to contain TMV in their leaves. On the other hand it was shown that such fully systemic infections are rather difficult to reproduce because even deliberate inoculation of roots sometimes fails to cause infection. Once root infection has established several factors determine its further development. Fulton (1941) demonstrated that the movement of the virus is at first predominantly downwards. It then depends on the age and susceptibility of the plant and the season whether the virus will be restricted to the root system or will eventually reach the aerial parts of the plant. It is thought that a blocking mechanism may exist in the stem as entry of the virus from the roots is sometimes arrested. Even where root infection does result in leaf symptoms there is a delay in the appearance of visible leaf symptoms compared with infection by inoculation of the foliage. Broadbent (1965a) found that the time taken for TMV to become systemic, i.e. detectable in the leaves by assay, from root infection varied from 3 to 24 weeks. The average time was 10 to 16 weeks and showed a tendency to increase with the age of the plants. The considerable variation in time observed among plants of the same age cannot be explained unless there are individual differences in susceptibility as suggested by Roberts (1950). A seasonal effect on the rate at which root infections become systemic is clearly indicated by the authors referred to, in particular Robers (1950). In an experiment carried out in the summer 10 out of 15 potted plants became infected in the roots within two months of the addition of infective sap to the soil. With 9 plants the root infections became fully systemic. In a similar experiment in the autumn of the same year again 10 out of 15 plants became infected in the roots, but only one plant developed leaf symptoms. In summarizing the experimental data available we may conclude that root

infection of tomato plants with TMV does occur to a limited extent and that the virus may become fully systemic.

The persistence of the virus in plant debris depends to a large extent on the moisture-air-relationships in the soil which determines whether conditions are aerobic or anaerobic. These in turn affect the number and kind of microorganisms responsible for the breakdown of infected debris and the subsequent disappearance of TMV from the soil. This natural inactivation proceeds at a rate far too slow to be of practical interest, because particularly in root debris the infectivity of TMV is preserved for a considerable period of time. Broadbent et al. (1965) still detected TMV in roots from a soil which had been left fallow for two years.

Steam sterilization is at present the best known method to inactivate TMV in the soil. To be effective the treatment should ensure a sufficiently high temperature reaching to a depth where tomato roots can penetrate. Temperature and time required to inactivate TMV depends on several factors including the nature and further conditions of the infected plant material and on the experimental methods (Table 1).

Broadbent et al. (1965) conclude that 20 minutes at 90°C is sufficient to inactivate TMV in fresh debris in soil, while Van Winckel & Geypens (1965) found at least 2 hours at 85°C necessary.

In general the usual methods of steam sterilization in commercial practice are not adequate because the temperatures achieved are not high enough or do not reach every part of the soil. From extensive temperature measurements by means of thermocouples Fletcher (1969) found irregularities in the distribution of temperatures to occur with all methods investigated. With the sheet method temperatures of 93°C were observed to a depth of 45 cm following a treatment of 8 hours. Similar measurements by Nederpel (1971a, b) however indicated that with this method the temperatures at the same depth often did not exceed 65°C and even at a depth of 30 cm remained below 80°C. Probably the differences in results obtained by the authors mentioned may be explained by differences in the depth of the soil above the water table. It should be pointed out that the efficiency of steam sterilization may depend on soil conditions like structure and texture of the soil, moisture content, etc. Promising experimental results were obtained with steam sterilization by means of a permanent system of drain pipes buried at a depth of 50 cm. This method allows for an evenly distributed temperature of 100°C from the depth mentioned to the surface of the soil. A temperature of at least 80°C was recorded at a depth of 60 cm.

The work of Broadbent et al. (1965) suggest that steam sterilization considerably delays TMV infection compared with soil left untreated or treated with chemical sterilants. Fumigation of soils with chemicals like chloropicrin, methyl isothiocyanate and others by killing off micro-organisms preserves plant debris and therefore its TMV contents. Van Winckel & Geypens (1965) report similar results with leaf sap as well as leaf fragments or stem pieces mixed with soil. With the latter material the authors noted an apparent increase of infectivity in the course of the experiment. Broadbent et al. (1965) observed the conserving action of chemicals on the TMV

Table 1. Inactivation temperatures for TMV in different plant materials.

Infected plant material	Temperature (°C)	Time of exposure (min)	Result of assay after treatment[1]	Author
Undiluted leaf sap	85[2]	<5	—	Broadbent et al. 1965
	88[2]	<5	—	
	85[2]	10 – 20	—	
	88[2]	10 – 20	—	
Leaf fragments mixed with soil	85[2]	≈60	—	Van Winckel & Geypens, 1965
	88[2]	<15	—	
Stem pieces mixed with soil	85[2]	480	+	
	88[2]	15 – 30	—	
Root pieces of 1–2 mm diameter				Broadbent et al., 1965
fresh	88[2]	10 – 20	—	
dried and stored for 4 months	82[3]	<5	—	
fresh	88[2]	15	—	Fletcher, 1969
dried and stored for 3 months	88[2]	15	—	
dried and stored for 5 months	88[2]	15	+	
Root pieces of 3–4 mm diameter				
fresh	82[2]	>15	—	
dried and stored for 3 months	93[2]	15	—	
dried and stored for 5 months	93[2]	15	+	
Root pieces of 5–7 diameter				
fresh	88[2]	15	—	
fresh	100[2]	15	+	

1. — = TMV not detected; + = TMV detected.
2. Temperature held constant in water bath.
3. Temperature obtained with air-steam mixtures.

content of roots and in addition demonstrated that as a result of chemical sterilization TMV infections occurred early in the crop.

2.4 Surface water as a primary source of inoculum

Van Dorst (1970) detected the presence of TMV in samples of surface water when using the very sensitive host plant *Nicotiana clevelandii* (Hollings, 1959). The concentrations of TMV involved were beyond the range of detection by the test plants normally used for assay like *N. glutinosa* of *N. tabacum* 'Xanthi nc'. He also found TMV to be present throughout the year. In view of the very infectious nature of TMV it is highly probable that surface water, which is generally used for watering tomato crops, might incidentally cause infections.

2.5 Discussion

The possibility of growing a tomato crop free from TMV should be considered against the background of commercial practice and trends in the development of cultural methods.

The data presented above on seed transmission and seed treatments suggest at least a reasonable chance for raising healthy plants on the nursery. Unfortunately, there are at present no seeds available certified to have been treated for virus, let alone virus free. The majority of seed growers are certainly not unwilling to apply some kind of treatment but often hesitate to do so because of undesirable side effects. The delay in germination observed following heat treatment at moderately high temperatures is one of the most serious disadvantages of this method. Trisodium phosphate often causes the seeds to turn a dull grey. To restore the original colour one of the seed growers (pers. commun.) bleaches with hydrochloric acid and so actually disinfects his seeds twice. But he is reluctant to publicize this treatment because he is well aware of the fact that they could be infected internally.

It should be noted that seed growers who can afford it employ an electronic scanning device to sort out entirely and partially necrotic seeds from lots graded already for size and specific gravity. Since necrotic seeds are usually infected internally and have poor germination (Broadbent, 1965b) the grading process indirectly reduces the risks of seed transmission of TMV. Graded seeds are mostly pelleted and sown singly further reducing the risk of infection from seed-borne TMV. Great care is often given to the germination quality of tomato seeds, but far less attention is paid to their virus contents.

On the other hand it should be realized that transmission of TMV also depends on methods used in raising tomato plants and it is in these methods that there are further opportunities for improvement. The soil of seed beds or benches on the nurseries of specialized plant propagators is usually not replaced or sterilized in between successive sowings and consequently an accumulation of contaminated seed coats throughout the season is almost unavoidable. While replacement of the soil or its sterilization would not be practicable a seed treatment with trisodium phosphate might be an acceptable alternative. A soak in this chemical when it is done first would only mean a minor adjustment to the practice of soaking seeds in water for one night prior to sowing.

After germination the seedlings are either pricked off first into small pots before being transplanted in larger ones or they are directly transplanted into large pots. The seedlings are often transplanted long after the stage when they are known to be less susceptible to infection. It is doubtful whether the growers would or could take advantage of this partially resistant stage by pricking off seedlings immediately following germination. Young seedlings are difficult to handle and do not show the defects for which they should be discarded later on. When large pots are used discarding plants would mean a substantial waist of potting soil especially with some hybrid varieties where defects are more common. In this connection the growers prefer to adopt the method of sowing pelleted seeds directly into small pots because tomato

plants raised this way sometimes give an earlier yield.

Since the best commercial methods of steaming often fail to inactivate TMV in the soil there appears to be little that can be done about this source. There are also circumstances when growers prefer to use chemical soil sterilants e.g. when a lettuce crop is grown before the main tomato crop and steaming is anticipated to cause manganese toxicity in the lettuce. This risk may be avoided with a system of drain pipes (Nederpel, 1971a and b) through which either steam or a steam-airmixture is applied dependent upon whether treatment at 100°C or 70°C is intended. It is evident that partial sterilization with a steam-air-mixture at approximately 70°C while controlling most of the harmful soil organisms and preventing the release of toxic amounts of manganese will not be effective against TMV. At 100°C the drain pipe method of steaming has definite advantages over the sheet method for the inactivation of TMV, although it will not produce effective temperatures at depths below 50 cm. However, it is questionable whether the buried pipe system can be made to work economically because it will be useful only for the purposes mentioned. Also the costs of installation at present are too high.

A widely different approach to the problem of preventing soil transmission of TMV has been suggested by Wheeler (1961) who proved that healthy tomato crops can be grown if roots are prevented from penetrating into the infected soil. This was accomplished when the tomato plants were grown in shallow beds with a peat-sand-mixture made on top of the infested soil but separated from it by polythene sheeting. Similar growing methods which by confining root growth induce a better fruit set would be quite acceptable especially for early winter crops, but would also require great skill on the part of the growers. To maintain well balanced growth the use of trickle irrigation is essential in order to apply critical amounts of water and fertilizer. Physiologic disorders such as blossom end rot often occur under these conditions. Probably only the most intelligent growers could profitably use such exacting methods of growing tomatoes.

2.6 Conclusion

Recent developments in cultural practice tend to create the conditions which make it feasible to grow a crop of tomatoes free from TMV. Features like sowing pelleted seeds directly into small pots and growing plants in plastic containers with the aid of trickle irrigation practically eliminate the chances of primary infections from seeds and soil respectively. Hygienic measures must be practised in order to prevent infection from other potential sources. Since the virus is spread mainly when handling the crop during cultural operations it is necessary for the workers to change cloths regularly and to disinfect hands and tools very frequently (Broadbent, 1964b). Skimmed milk preparations used as a dip for hands and tools might prove an efficient yet harmless means of checking spread of the virus (Jaeger, 1966) whenever the disease occurs.

The average grower can hardly be expected to put these measures into practice unless he is fully convinced that they will increase his profits. With painstaking care

he may succeed in growing a healthy crop of tomatoes but the returns in yield may not compensate for extra cost of the labour. There is also the doubt that after all his effort the precautions taken will not be adequate when considering the very infectious nature of TMV.

In this situation the grower might prefer to use methods to give a general improvement in the cultural environment enabling him to avoid the worst damage of infection. Climatic control systems can be used to minimize losses resulting from poor fruit set since temperature and relative humidity can be kept at the optimal level.

In conclusion preventive control of TMV although not wholly impracticable will remain a risky undertaking and may not be worth the effort.

3 Strains of tobacco mosaic virus on tomato in the Netherlands as distinguished by symptom expression

3.1 Introduction

Workers engaged in the problem of TMV in tomatoes have always been impressed by the bewildering variability of its symptoms, which was taken as evidence for the existence of different strains of the virus. The strains isolated were often described with reference to the characteristic symptoms found in the original plant material. Such descriptive names were adequate provided the symptoms observed could be reproduced but caused a great deal of confusion when this was not the case. A mosaic pattern on the leaves in dark and light shades of green was ascribed to a common strain because this symptom occurred most frequently. For a long time it was believed that this common strain was identical to ordinary tobacco mosaic virus (Ainsworth, 1933). This assumption was based on the study of too small a number of mosaic samples. It was not before host plants had been found to differentiate the green mosaic strains (Kassanis & Selman, 1947), and Broadbent (1962) and others had made extensive surveys, that the situation became clear. It then became obvious that the ordinary tobacco mosaic virus was, in fact, quite uncommon in tomatoes. The strain which in reality had to be regarded as common had been named the tomato streak virus as it had been found associated with rather exceptional necrotic symptoms. So two strains, while distinct in their reaction on appropriate test plants, may still cause similar symptoms on tomato and, conversely, one strain may cause various symptoms on the same host. It is at present generally accepted that symptoms represent only the visible expression of the interactions between virus and host as affected by the environment. For a description of TMV strains on tomato it is therefore not enough to mention only the symptom characteristics on this particular host. It is also necessary to indicate the conditions essential for the symptoms to appear and to establish either biologically or otherwise the true TMV nature of the strains involved.

It is the scope of this chapter to present a survey of TMV strains in tomato in the Netherlands and to compare them with similar strains found and described elsewhere. The strains have been provisionally classified according to the symptoms produced on tomato, tobacco and additional test plants. This is done with the knowledge that any such classification may be liable to criticism. However, the description of strains given below is meant as a guide to their recognition in glasshouse crops of tomatoes rather than as a key for their identification in the laboratory. Meanwhile, it was inevitable to include a discussion on the elusive phenomenon of 'streak' since it has been used in strain classification and may be connected with necrosis strains.

3.2 The isolation of strains of TMV

While TMV in tomato usually occurs alone, at times its isolation may be complicated by the presence of 'alien' viruses like potato virus X (PVX). When a sample of plant material with necrosis was thought to contain TMV and PVX it was pretreated either with heat or with ethanol. For heat treatment a test tube containing crude sap was held immersed in a water bath for 10 minutes at 75°C in order to eliminate PVX (MacNeill & Ismen, 1960). The ethanol treatment (Henderson Smith, 1928b) consisted of grinding plant material in a mortar with 96% ethanol, allowing it to stand for 1 hour at room temperature and then pouring the suspension over a piece of filter paper in a petri dish to allow the ethanol to evaporate. The inoculum was prepared from the dried filter paper by grinding it with some water. When inoculated to tomatoes necrosis never resulted and this was considered sufficient proof that PVX had been eliminated from the mixture.

There is no doubt about the fact that TMV occurs naturally in a mixture of strains and several techniques are used for their isolation. The one generally applied is the so-called 'single lesion' method where local lesions, which develop on the inoculated leaves of a hypersensitive type of assay host like *Nicotiana glutinosa*, are used (Johnson, 1947). It is necessary to apply the inoculum in a number of dilutions in order to find the optimum concentration to give well spaced lesions. The selected lesion is cut or punched out and after being ground with a droplet of water used for the inoculation of a systemic host. The utensils required for the transfer of 'single lesions' are shown in Fig. 1.

Another method of isolation, which was occasionally used by the author is known as the 'single pin prick' method (Holmes, 1928). This enables the transmission of virus from small confined areas with symptoms that are different from the surrounding diseased plant tissue. The only tool required is a fine insect pin mounted on a handle or held with pincers. It is first pricked into the target area e.g. a yellow fleck on a leaf with a predominant green mosaic pattern and then into parts of a young systemic host, preferably near the leaf veins. Since the rate of transmission by this method was usually very poor it was resorted to only when the 'single lesion' method appeared impracticable.

Several test plants were used in connection with the above mentioned isolation techniques. *N. glutinosa* or *N. tabacum* 'Xanthi nc' were used as local-lesion hosts, whereas *N. tabacum* 'Samsun' generally served as a systemic host for the multiplication of virus. The choice of test plants used was adapted according to the isolate e.g. *N. tabacum* 'White Burley' was preferred as a systemic host to 'Samsun' for the brighter yellow colour of the symptoms following an infection with yellow strains. When a sample showed no particularly interesting symptoms single lesions were used to inoculate a host for virus multiplication and also one for the differentiation of the tobacco and tomato strains of TMV. The 'White Burley' selection referred to as 'Dutch A' (Broadbent, 1962) or the 'necrotic' line (Termohlen & Van Dorst, 1959) was used for this purpose. Usually the 'single lesion' isolation had to be repeated several times until

a consistent pattern of symptom development was observed on systemic hosts. With isolates from tomato fruits attempts were made to reproduce the original fruit symptoms by inoculating tomato plants at a time when the first fruits had set.

Many solanaceous species have been tested in a search for additional hosts for the further separation of TMV strains. The few interesting species will be indicated at the appropriate places.

Before passing on to the description of the symptomatological strains isolated it is necessary to give a definition of the term 'isolate' and 'strain'. In accordance with the Dutch list of phytopathological terms (Lijst van plantenziektenkundige termen, 1968) the term 'isolate' will be used to denote a pure culture of TMV obtained by the single-lesion method. It is realized, however, that the purity of any isolate may be questionable as long as it is impossible to start a virus culture from a single particle. The term 'strain' is used here to denote a group of isolates with a certain similarity in symptom expression or host range. In view of the considerable symptom variation among isolates the distinction of such strains has been restricted to a minimum.

3.3 Strains of TMV distinguished by symptoms

3.3.1 Tobacco and tomato strains

Ordinary tobacco mosaic virus and the virus usually found in tomato show only minor differences in their symptom expression on systemic hosts. Because they can be differentiated on additional test plants it is proposed to call them the tobacco and the tomato strain of TMV after the host plants they are adapted to. The mosaic pattern caused by the tobacco strain on tobacco leaves is mostly accompanied by characteristic blister-like malformations which are more severe during winter months. These malformations never appear with the tomato strain. On the other hand, senescent leaves of systemically infected tobacco plants will seldom show necrosis unless the tomato strain is involved.

Mosaic symptoms on tomato leaves are the same for the tobacco and tomato strains. However, leaf malformation known as 'fern leaf' which develops during the early stage of an infection under poor light conditions seldom fails to appear with the tomato strain, but does not always occur with the tobacco strain. When the tobacco strain is involved the malformation affects the leaves in an irregular way. The tobacco and the tomato strain can be shown to be biologically distinct by the reactions of a number of differential hosts (Table 2).

The tobacco strain invades most of the differential hosts systemically whereas the tomato strain is confined in local necrotic lesions (see Fig. 2). It is remarkable that the reverse set of reactions is observed on *Solanum giganteum*.

Many surveys have been made to assess their relative importance in tomato crops and it was established that the tomato strain occurred predominantly (MacNeill, 1962; Broadbent, 1962; Komuro et al., 1966; Van Winckel, 1967). Similarly I found

Table 2. List of non-differential and differential host plants for tobacco and tomato strains of TMV. L = local necrotic lesions, S = systemic mosaic.

Host plant	Host reaction	
	tobacco strain	tomato strain
Non-differential:		
Nicotiana glutinosa	L	L
Nicotiana tabacum 'Xanthi nc'	L	L
Nicotiana tabacum 'Samsun NN'	L	L
Nicotiana tabacum 'Samsun'	S	S
Nicotiana tabacum 'White Burley'	S	S
Petunia nyctagyniflora[1]	S	S
Differential:		
Nicotiana rustica	S	L
Nicotiana sylvestris	S	L
Nicotiana tabacum 'White Burley'[2]	S	L
Petunia hybrida	S	L
Petunia nyctagyniflora[1]	S	L
Physalis ixocarpa	S	L
Solanum giganteum	L	S

1. Lines originating from seeds kindly supplied by Dr Schade, Martin Luther Universität, Halle, DDR.
2. 'Necrotic' line or 'Dutch A'.

175 out of 200 tomato-leaf samples collected in 1964 from all over the country to contain only the tomato strain. The remainder of the samples contained both strains of the virus. An explanation for the dominance of the tomato strain in tomato crops is suggested by experimental results which indicate a faster rate of multiplication or a greater invasiveness of the tomato strain in tomato compared with the tobacco strain (Komuro et al., 1966; Jensen, 1968; Tomaru et al., 1970). I studied the result of the interference between the tomato and tobacco strains one month after their simultaneous or successive introduction into tomato plants of different age. A period of one month was allowed to elapse between the first and second inoculation. The effect of the inoculations was evaluated by means of the single-lesion technique. Tip leaves of the tomato plants were sampled one month after the only inoculation made or after the latest of two inoculations. For each treatment 100 lesions produced on *N. glutinosa* were transferred singly to plants of the 'necrotic' line of *N. tabacum* 'White Burley' for the differentiation of the tomato and tobacco strain of TMV. The experiment was repeated four times.

From the results presented in Table 3 it is apparent that there is no absolute cross protection between the tomato and tobacco strains and that they can coexist in tomato. This result confirms the earlier work of MacNeill & Ismen (1960). Furthermore the dominance of the tomato strain is well illustrated in Treatments 2 and 5

Table 3. Interference of tomato and tobacco strains of TMV in tomato. Number of lesions from *Nicotiana glutinosa* causing a local (L), mixed (M) or systemic (S) reaction on the 'necrotic' line of *N. tabacum* 'White Burley' indicating the presence of the tomato strain, the tobacco strain or both strains respectively.

Treatment	Experiment 1 April-June 1965			Experiment 2 July-Sept. 1965			Experiment 3 Oct.-Dec. 1965			Experiment 4 Jan.-March 1966		
	L	M	S	L	M	S	L	M	S	L	M	S
1. Tomato strain inoculated on cotyledons of seedling plants, tobacco strain inoculated on true leaves a month later	69	23	8	100	0	0	98	1	1	64	14	22
2. Tobacco strain inoculated on cotyledons of seedling plants, tomato strain inoculated on true leaves a month later	41	53	6	76	14	10	76	12	12	48	32	20
3. Tomato and tobacco strains inoculated each on cotyledon of seedling plants	97	3	0	87	9	4	56	33	11	98	2	0
4. Tomato strain inoculated on true leaf of tomato plants a week before flowering, tobacco strain inoculated a month later	92	7	1	66	25	9	88	12	0	73	17	10
5. Tobacco strain inoculated on true leaf of tomato plants a week before flowering, tomato strain inoculated a month later	46	36	18	66	19	15	70	30	0	52	29	19
6. Tomato and tobacco strain inoculated separately on successive true leaves of tomato plants a week before flowering, tomato strain on lower most leaf	36	45	19	85	15	0	85	11	4	22	65	13
7. Tomato and tobacco strain inoculated separately on successive true leaves of tomato plants a week before flowering, tobacco strain on lower most leaf	37	27	36	89	9	2	85	10	5	56	39	5

where recovery of the tobacco strain decreased in incidence. All treatments except 3 and 4 suggest that the season may affect competition between the tomato and tobacco strain. The tomato strain appears to be favored in conditions with decreasing day length. From the relationships between the tobacco and the tomato strain in tomato it may be concluded that smoking tobacco which usually contains the tobacco strain is not at present an important source of TMV for commercial tomato crops. This is in accordance with the experience of previous authors (Broadbent, 1962; Komuro & Iwaki, 1968).

3.3.2 The tomato enation strain

The tomato enation strain (Ainsworth, 1937) is characterized by enations or leafy outgrowths on the underside of tomato leaves showing the 'fern leaf' symptom (Fig. 3). It was isolated on rare occasions from a single plant or a small number of plants showing a severe leaf narrowing in a crop with normal mosaic symptoms. On young tomato plants the fern leaf symptoms caused by this enation strain do not differ from those caused by the tomato strain in winter time. In contrast with the tomato strain the enation strain causes this kind of malformation independently of the season. It also affects the shape of flowers accounting for the abnormally poor setting of fruits and therefore heavy losses in yield (Rast, 1967a). On some systemic hosts the enation strain may cause malformations not unlike those of the tobacco strain, but the reactions of differential hosts show its affinity to the tomato strain.

3.3.3 The yellow mosaic or aucuba strain

There is a considerable variation in symptom expression among TMV isolates which cause yellow discolorations on tobacco or tomato. Work by Jensen (1933) suggests that each one of these isolates may well represent a distinct strain. The isolate which is recognized as the yellow mosaic or aucuba strain may not be identical to the strain described by former authors (Henderson Smith, 1928a; Ainsworth et al., 1934; Kunkel, 1934) in spite of a close resemblance of symptoms. It is certainly not identical to the 'flavum' strain (Friedrich Freksa et al., 1946) which like the tobacco strain it was derived from produces systemic symptoms on *N. sylvestris*. The yellow mosaic strain occurs sporadically in tomato crops where it usually infects only a small number of plants often in a single row dependent on the presence of the tomato strain which checks its further spread. During the initial stage of an infection the inoculated leaflets of young tomato plants show bright yellow, primary lesions which rapidly become necrotic. Systemically infected leaves, which following a transient vein-clearing turn completely yellow may later become severely necrotic. In a later stage the leaves develop a yellow mosaic pattern together with some distortion and occasional necrotic spots. The yellow colour turns almost white with increasing age of the leaves. All other parts of the plants are affected in a similar way and a more or less

Fig. 1. The single-lesion method of isolation. A lesion punched out from a 'Xanthi nc' tobacco leaf is freed from the punch (diameter 1.5 mm) with a mounted needle and applied to a drop of water on an object glass. It is afterwards macerated with one of the glass spatulas on the right and inoculated to a systemic host. The bottle of alcohol (ethanol) and spirit burner on the left are used for flame-sterilizing punches, needle and pincers used to press the leaf flat before punching out lesions.

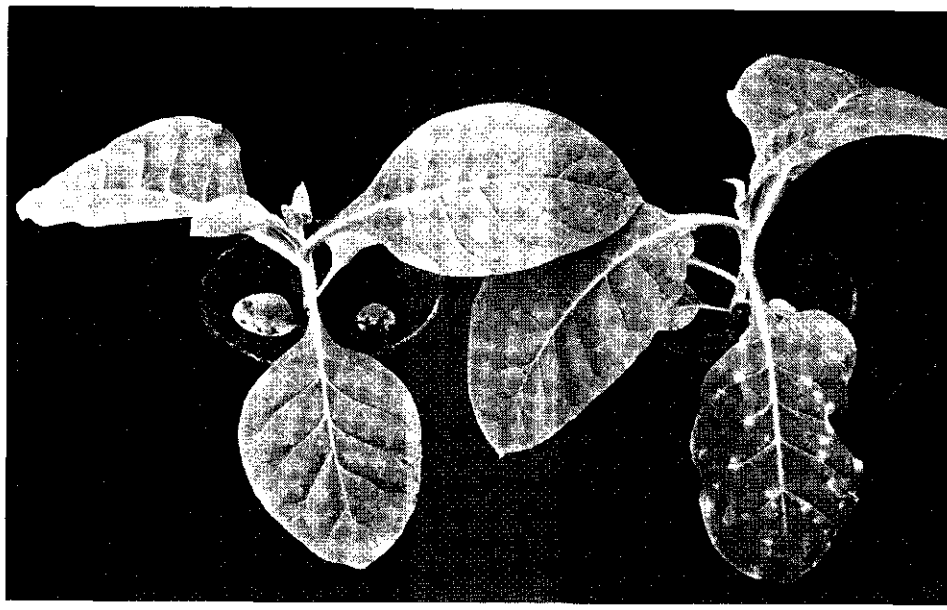

Fig. 2. Plants of the 'necrotic' line of *Nicotiana tabacum* 'White Burley' about one week after inoculation with the tobacco strain (left) and the tomato strain of TMV (right). Note the necrotic local lesions caused by the latter.

Fig. 3. Tomato leaflets malformed by the tomato enation strain and showing enations on their undersides. One of the leaflets is shown in more detail (isolate NV).

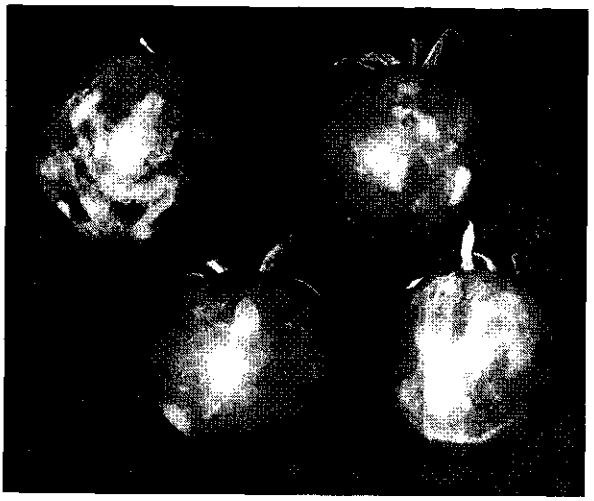

Fig. 4. Tomato fruits with yellow flecks caused by the yellow mosaic strain (isolate GM).

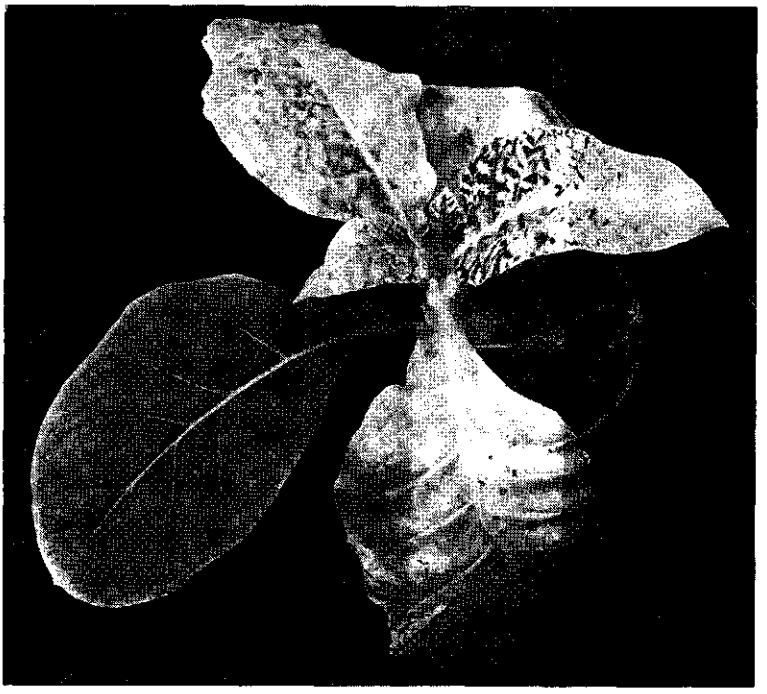

Fig. 5. A plant of the 'mosaic' line of *Nicotiana tabacum* 'White Burley' showing the mosaic pattern caused by the yellow mosaic strain (isolate SL^c).

Fig. 6. Tomato leaflets showing vein yellowing and isolated ringspots caused by the yellow ringspot strain (isolate SG-64).

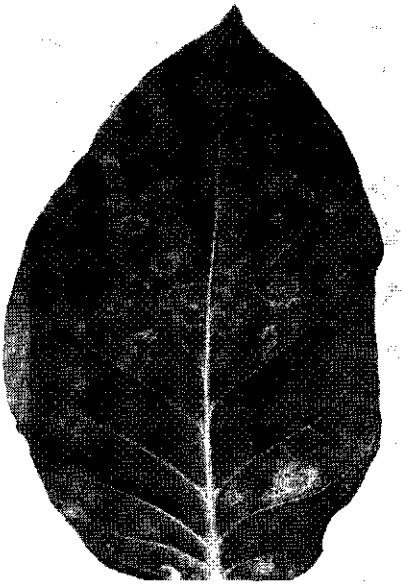

Fig. 7. Leaf of *Nicotiana tabacum* 'Samsun' with ringspots caused by the yellow ringspot strain (isolate GvdD).

Fig. 8. A leaf of *Nicotiana glauca* showing systemic ringspots caused by the yellow ringspot strain (isolate GdK).

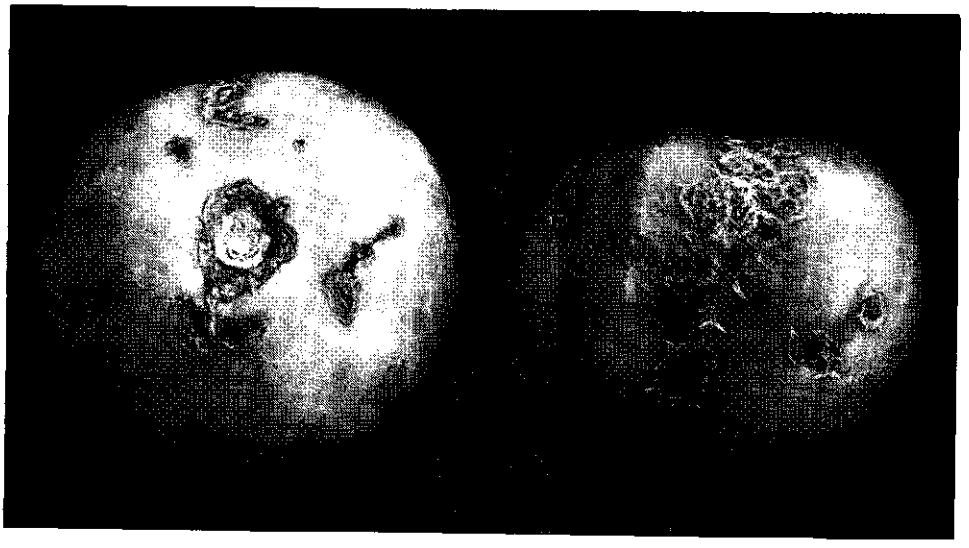

Fig. 9. Tomato fruits with patches of corky tissue caused by the tomato crusty fruit strain (isolate MKv). Note that the tissue around the calyx is also affected.

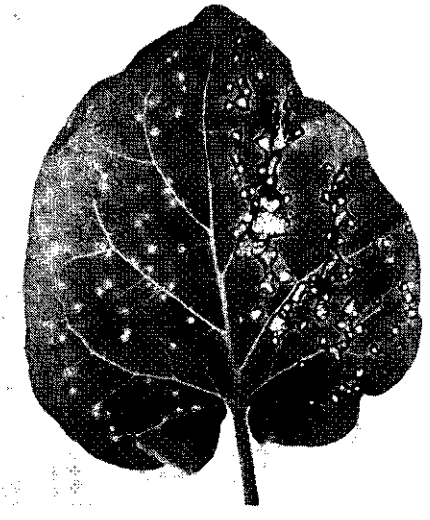

Fig. 10. Leaf of *Nicotiana glutinosa* showing lesions of normal size on the left half caused by the tomato strain (isolate SPS) and abnormal small lesions on the right half caused by the crusty fruit strain (isolate MKv).

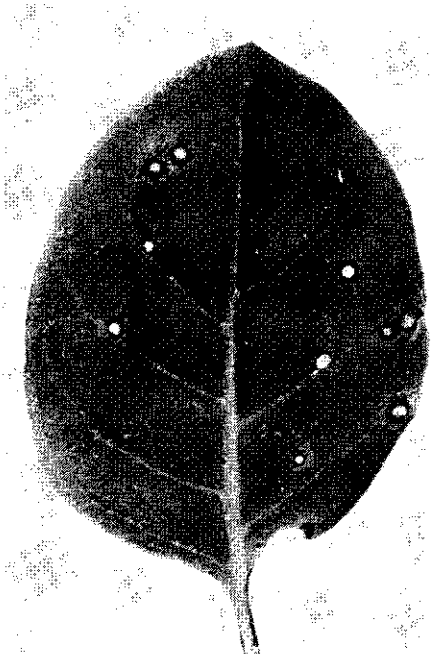

Fig. 11. Leaf of *Nicotiana glauca* with local necrotic lesions caused by the winter necrosis strain (isolate SL[a]).

Fig. 12. Desiccative effects of the winter necrosis strain (isolate SL[a]) on a shoot of tomato in winter time.

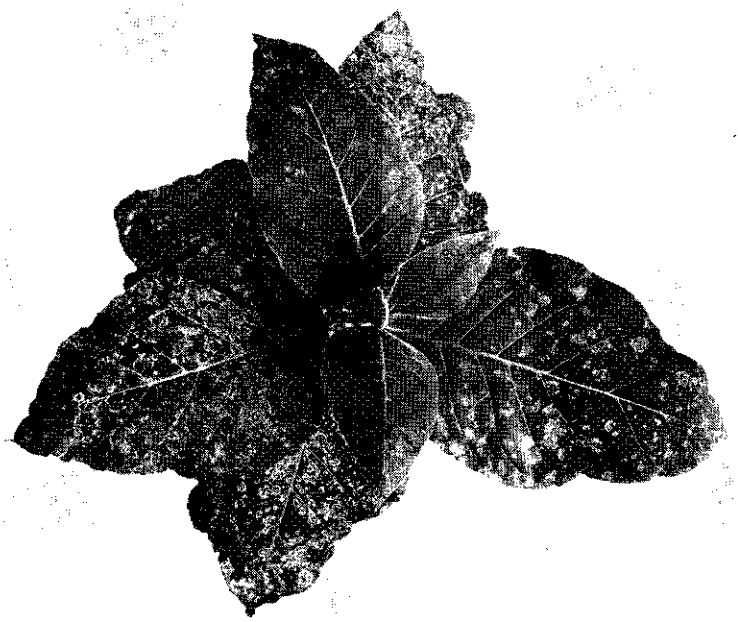

Fig. 13. Necrotic ring patterns caused by the winter necrosis strain (isolate SL[a]) on *Nicotiana tabacum* 'Samsun' in autumn.

Fig. 14. Necrotic streaks along stem and leaf stalks of tomato caused by the summer necrosis strain (isolate SBK).

severe mottle of the fruits (Fig. 4) makes them worthless (Rast, 1967a). A general effect of infection is severe stunting of growth. Similar symptoms are produced on other systemic hosts (Fig. 5). On differential hosts the reaction caused by the yellow mosaic strain is typical for the tomato strain of TMV.

3.3.4 The tomato yellow ringspot strain

The yellow ringspot strain described in an earlier paper (Rast, 1965) may be considered representative of many isolates characterized by yellow ringspot symptoms on tomato and tobacco (Fig. 6 and 7). Systemic yellow ringspots on *N. glauca* (Fig. 8), however, have so far been observed with only two isolates. Among the strains reported in literature the yellow ringspot strain bears a superficial resemblance to the 'luridum' strain of Friedrich Freksa et al. (1946). When chlorosis is seen in a tomato crop the yellow ringspot strain is generally involved. Contrary to the striking effects of the yellow mosaic strain its symptoms are relatively mild. Leaf symptoms consist of a pale yellow mosaic which on close examination reveals ringlike patterns. Characteristic scattered ringspots or ring effects produced by vein-yellowing may be found particularly on fully expanded older leaves. Necrosis is virtually absent. Fruit symptoms may vary from hardly visible yellowish spots to distinct concentric rings. Growth rate of the plants is not perceptibly affected especially when compared with the serious stunting in growth caused by the yellow mosaic strain. Observations in commercial crops suggest that the appearance of symptoms may be closely connected with relatively high temperatures e.g. when symptoms are confined to plants growing in the blast from a oil-burner used for CO_2 enrichment and other similar 'hot spots' occurring in glasshouses. The yellow ringspot strain occurs rather frequently but its economic importance is difficult to assess since it apparently causes no substantial loss in yield (Rast, 1967a). On certain hosts the yellow ringspot strain causes the same type of reaction as the tomato strain of TMV.

3.3.5 The tomato crusty fruit strain

Fruit symptoms caused by four isolates of the yellow ringspot strain may justify their classification as a distinct strain. The symptom characteristics of these four isolates have not previously been described. One isolate causes a brilliant yellow mosaic with ring patterns on the leaves of tomato. On the fruits this isolate causes superficial, corky tissue to develop forming incomplete or closed rings. As the result of differences in growth rate the fruits may burst open along the lines of corky tissue or may show a blistery appearance. Another isolate is characterized by some systemic necrosis along the veinlets of young unfolding tomato leaves. The necrotic dots do not extend in size and seem to vanish with increasing age of the leaves. Fruit symptoms also consist of corky crusts which hardly affect the shape of the fruits (Fig. 9). The isolate is characterized by abnormally small, local lesions on *N. glutinosa* and *Solanum persicum* (Fig. 10). The remaining two isolates produce yellow ringspots on leaves of tomato

and tobacco, and also corky crusts on tomato fruits. They also produce minute local lesions on *N. glutinosa*. The fruit crusts described may be easily mistaken for scars resulting from mechanical damage. It is proposed that these should be classified as the crusty fruit strain as this symptom is the only one characteristic of all four isolates.

3.3.6 Necrosis strains

Strains of TMV, which cause necrosis on systemic hosts, are extremely rare. One of them is apparently related to the strain described by Doolittle & Beecher (1942) as 'Marmor tabaci var. siccans' which apart from severe necrosis on tomato causes local necrotic lesions on *N. glauca* (Fig. 11). These authors made no mention of seasonal influences upon the expression of symptoms, although the strain studied in this work shows systemic necrosis on tomato from late autumn to early spring. An inoculation during this period is lethal to young tomato plants. When fruit-bearing tomato plants are inoculated necrosis develops on the terminal leaves and along stems and leaf stalks. Terminal and side shoots as well as the petioles of older leaves may be constricted at their bases by this necrosis and as a result wither and die (Fig. 12). Flower and fruit trusses are affected in a similar way, immature fruits developing irregular, sunken necrotic blotches. In the remaining part of the year this necrosis strain causes much the same symptoms as the tomato strain; no significant differences in yield were observed in an experiment in which both strains were compared (Rast, 1967a). The leaf symptoms also vary with the season particularly on 'Samsun' tobacco. The diffuse chlorotic ringspots that appear in the green mosaic pattern in summer are markedly necrotic in winter (Fig. 13).

The same seasonal effect upon symptom expression was observed with an isolate obtained from abnormally light-coloured lesions on *Petunia hybrida*, which in turn had been inoculated with a single-lesion isolate derived from the necrosis strain. This isolate had lost the property to cause necrosis on tomato and local necrotic lesions on *N. glauca*. During the winter the symptoms on 'Samsun' consisted of necrotic lesions on inoculated leaves. From these lesions systemic necrosis started to develop slowly along the stem and main veins of higher leaves. Sometimes together with necrotic oak leaf patterns and rings. The symptoms in summer are not distinct from those caused by isolates of the yellow ringspot strain. It should be pointed out that inoculum prepared from systemically infected leaves, which have been collected and dried in summer, will not produce local necrotic lesions on 'Samsun' in winter, although it may still be infective. If leaves with necrotic lesions produced in winter are dried, infectivity is soon completely lost. Reproduction of this symptom was only possible with plant material, particularly stems, which had been kept in the deep-freeze box through summer. The trouble in maintaining an isolate which produced local necrotic lesions during winter time was also reported by Jensen (1937) with his strain no. 14. It is possible, that the present isolate is either a mixture of strains or a highly unstable necrosis strain which may give rise to other strains by mutation (Norval, 1938).

Another strain from tomato shows the opposite seasonal behaviour with necrosis

during the summer when glasshouse temperatures at times may well exceed 30°C. In these conditions symptoms on young tomato plants consist of primary yellow lesions and a systemic vein yellowing which is almost immediately followed by necrosis on leaves and along stems and leaf stalks (Fig. 14). Plants may be killed by stem necrosis, which is sometimes the first symptom to appear on fruitbearing plants. Plants which recover from the worst effects remain stunted in growth and develop a yellow mosaic with ring patterns on the leaves. Immature fruits may become malformed as a result of yellow ring formation preventing the development of affected tissue. The symptoms on 'Samsun' and other systemic hosts are essentially similar, also less severe than on tomato. In the remaining part of the year when necrosis is not particularly striking the present strain behaves as an isolate of the yellow ringspot strain. It is, however, different from the latter as it causes local necrotic lesions on *Solanum giganteum* and *S. villosum* prior to systemic symptoms. Also it differs from the 'tomato streak strain' described by Komuro (1963) because with the latter strain necrosis on tomato apparently appear throughout the year. On the other hand both strains cause local necrotic lesions on *S. giganteum*. The lesions on *S. giganteum*, unlike those caused by the tobacco strain of TMV, are not of the hypersensitive type.

On other differential hosts necrosis strains show a reaction typical of the tomato strain of TMV. Since necrosis is obviously linked with season it is proposed to call the strains causing this symptom on tomato the winter necrosis strain and the summer necrosis strain respectively. The unstable behaviour of the isolate characterized by necrotic lesions on 'Samsun' in winter does not justify its classification as a strain.

3.4 The phenomenon of 'streak'

Either TMV alone or both TMV and PVX may be isolated from tomato plants with necrosis on stems and leaf stalks. This necrosis is popularly known as 'streak'. A distinction is therefore made between 'single virus streak' and 'mixed virus streak' or 'double virus streak'. Reproduction of necrosis is seldom accomplished with TMV alone but is readily obtained when both viruses are present. In commercial tomato crops affected by 'single virus streak' the necrosis remains confined to a relatively small number of plants which are scattered throughout the crop. By contrast 'mixed virus streak' spreads rapidly in the direction followed by workers during cultural operations, often affecting whole rows of plants or even all the crop. Usually the strain of TMV isolated from plants affected by 'single virus streak' subsequently causes normal mosaic symptoms like those of the tomato strain. Early reports by Jarrett (1930), Ainsworth et al. (1934) and others on the experimental reproduction of necrosis are questioned by MacNeill & Ismen (1960) who suggest that PVX may not have been eliminated from the inoculum. These authors, like Vanterpool (1926), concluded that 'streak' was exclusively caused by the combined action of TMV and PVX after they failed to produce other than mosaic symptoms with inoculum treated for 10 minutes at about 70°C. So far, no conclusive evidence has been presented to oppose this view.

There exist a strong suggestion that in 'single virus streak' infection by TMV merely acts as a trigger mechanism eliciting necrosis from plants already disposed to react in that way by other factors. Among them climatic factors are probably the most important as the incidence of the disease is mainly limited to the winter and early spring months. Observations in winter crops suggest that a sudden cooling of plants e.g. by a failure of the heating system or by watering plants with icy-cold water with overhead-sprinklers, may induce 'streak' symptoms. Apart from these accidental 'cold shocks' temperature effects may be implicated when affected plants are found just below the ventilators or in parts of the glasshouse where temperatures are constantly below the desired level. These observations are supported by the experimental results of Wlassow (1962) who claimed a hundred percent reproduction of 'single virus streak' with plants exposed to a temperature between 16 and 18°C and a light intensity between 40 and 75 $J \cdot m^{-2} \cdot sec^{-1}$. However Rast (1970) using controlled environment cabinets failed to reproduce necrosis with isolates of the tomato strain originating from 'streak' affected tomato plants. In this experiment 60 different climates were tested by combining temperatures of 15, 18, 21 and 24°C with day lengths of 8, 12 and 16 hours, with the light intensity at bench level being approximately 50 $J \cdot m^{-2} \cdot sec^{-1}$. It was remarkable that in this experiment done in the winter necrosis occurred only with the winter necrosis strain and at practically all temperature-day length combinations tested. The temperatures were probably too low for the summer necrosis strain to induce necrosis. The plants infected with this strain mostly produced mild green mosaic symptoms. However, a bright yellow mosaic appeared when the plants were exposed to a day length of 16 hours with day and night temperatures in the first week of 21 and 15°C respectively and 24 and 18°C in the second week after inoculation.

Another important factor which may be involved with 'single virus streak' is root damage caused mechanically or otherwise. The disease is often connected with events early in the history of the crop like rinsing soil off the roots of seedlings before potting them or soaking potted plants in water so that they recover from wilting before planting out. Loss of roots occurred on 'streak' affected plants where the potting soil had been removed before they were planted in gravel beds. In an attempt to reconstruct the latter case in gravel culture Rast (1968b) obtained severe 'streak' symptoms on 33 out of 96 plants which had been stripped of practically all roots. Only 3 plants out of a further 96 where the roots were not damaged developed some leaf necrosis, the remainder of the plants in each treatment showing normal mosaic symptoms. Unfortunately, as the plants used in this preliminary experiment were neither assayed nor inoculated, the source and time of the natural infection is unknown. Although not showing leaf symptoms at the start of the experiment some of the plants may have been infected in their roots. As the incidence of 'single virus streak' in commercial tomato crops is often associated with a late infection it may originate from root infections, which are known to result in a delayed expression of leaf symptoms (Broadbent, 1965a). Experimental root inoculations at different times in combination with various other treatments failed to produce 'streak' symptoms in a normal soil culture (Rast, 1968b).

A third factor which is probably involved in 'single virus streak' is the variability of TMV itself as a potential source of necrosis inducing strains. While it is true that the tomato strain is isolated most of the time it is not necessarily the only strain present. Actually, one sample of 'streak' affected leaves may contain two or more strains including the tomato yellow ringspot strain and the tobacco strain. Samson (1942) found a strain causing yellow ringspot symptoms in association with 'single virus streak'. Occasionally isolates were obtained which caused only local necrotic lesions on 'Samsun' like those described previously for the isolate derived from the winter necrosis strain. Such isolates mostly ceased to incite necrosis after one or two transfers. On the other hand, of the many isolates derived from strain no. 14 (Jensen, 1937) one was observed to kill young tomato plants (Norval, 1938).

The apparent instability shown by these necrosis inducing isolates suggest that they may represent defective strains which for lack of a protective protein-coating are rapidly inactivated outside living plant tissue (Kassanis & Woods, 1969). This would account for the fact that 'streak' symptoms may be transmitted by grafting but not by mechanical inoculation (Van Dorst, 1963). Combining the observations in commercial practice and the experimental data it is evident that a complex of factors is involved in 'single-virus streak'. I doubt whether this fascinating problem will ever be solved, not because of the difficulties in reaching its solution, but rather because its small economic importance will not warrant further investigations.

3.4 Discussion

The classification of TMV strains on the basis of symptoms may seem a precarious matter in view of the fact that one strain may yield many others (Jensen, 1933) and that symptoms may vary considerably with environmental conditions. A notorious example for the latter is the 'internal browning' symptom of tomato fruits which was connected with the ribgrass strain (Holmes, 1950) although it is now known to be primarily an effect of late infection rather than a specific strain of TMV (Boyle & Wharton, 1957; Smith et al., 1965). Similarly, in most cases of 'single virus streak' there is no causal relationship between the strain found associated with it and the necrotic symptoms. So, it is clear that before naming a strain after symptoms it must be established that TMV is the cause of the symptoms and that the strain will also produce these symptoms. If these conditions are satisfied there is no reason why a descriptive name should not be used, especially when it is meant for local use only or for the study of practical objectives. It is with these reservations that the classification as presented in Table 4 should not be regarded as final, but rather as a preliminary working scheme. The use of further biological or chemical criteria will, no doubt, enable the separation and recognition of more strains. Such separation may make it more difficult to assess their possible practical significance.

There is no doubt that at present the tomato strain of TMV is dominant in tomato crops. Early investigations (Ainsworth, 1933) suggested that the tobacco strain was dominant which lead Broadbent (1961) to consider whether the tomato strain evolved

Table 4. Survey of TMV strains as occurring in glasshouse crops of tomatoes in the Netherlands.

Strains	Symptom characteristics of systemic hosts	
	Tomato cv. 'Moneydor'	Tobacco cv. 'Samsun'
Tobacco strain	green mosaic	green mosaic leaf distortion
Tomato strain	green mosaic, leaf distortion in winter	green mosaic, some necrosis on s nescent leaves
Tomato enation strain	green mosaic, leaf distortion	green mosaic, leaf distortion
Tomato yellow mosaic strain	bright yellow mosaic, necrosis in initial stage of infection, yellow spots on fruits	bright yellow mosaic, necrosis initial stage of infection
Tomato yellow ringspot strain	pale yellow mosaic with ringspots, variable degree of ringspotting of fruits, no necrosis	pale yellow mosaic, distinct ring pots, no necrosis
Tomato crusty fruit strain	pale to bright yellow mosaic with ringspots, corky crusts on fruits	pale to bright yellow mosaic, distin ringspots
Tomato winter necrosis strain	green mosaic in summer, severe necrosis in winter	green mosaic, distinct necrotic ring pots in spring and autumn
Tomato summar necrosis strain	yellow mosaic with ringspots in winter, severe necrosis in summer	yellow mosaic with distinct ring pots, necrosis in summer

eaction of tobacco cv. /hite Burley' ('necrotic ıe')	Reaction of additional test plants	Strains compared to in literature
stemic	no reaction on *N. glauca*, local necrotic lesions on *S. giganteum* (Table 2)	Tobacco mosaic virus (for synonyms see K. M. Smith, 1957)
cal	no reaction on *N. glauca*, systemic mosaic on *S. giganteum* (Table 2)	Petunia local-lesion virus (MacNeill, 1962); Tomato glasshouse streak virus (Ainsworth, 1933); further synonyms K. M. Smith (1957)
cal	no reaction on *N. glauca*, systemic on *S. giganteum*	Tomato enation mosaic virus (Ainsworth, 1937)
cal	local yellow lesions on *N. glauca*, systemic on *S. giganteum*	Tomato yellow mosaic virus (Henderson Smith 1928a); Marmor tabaci var. flavum (Friedrich-Freska et al., 1946)
cal	systemic yellow ringspots on *N. glauca* (2 isolates), systemic on *S. giganteum*	Marmor tabaci subsp. dahlemense var. luridum (Friedrich-Freksa et al., 1946)
cal	minute local lesions on *N. glutinosa* and *S. persicum*, no reaction on *N. glauca*, systemic on *S. giganteum*	
cal	local necrotic lesions on *N. glauca*, systemic mosaic on *S. giganteum*	Marmor tabaci var. siccans (Doolittle & Beecher, 1942)
cal	no reaction on *N. glauca*, local and systemic necrosis on *S. giganteum* and *S. villosum*.	Tomato streak strain (Komuro, 1963)

from the tobacco strain. This seems unlikely as the 'fern leaf' symptoms described by Westerdijk (1910) are suggestive for the tomato strain. Furthermore, the four TMV isolates examined by Ainsworth et al. (1934) two of which were tomato and two tobacco strains may not have been a true reflection of the proportion of these strains found in crops at that time. It seems more likely that they reflected the authors' interest in isolates associated with symptom types, particularly 'streak'. Thirteen out of fifteen 'streak' samples yielded the tomato strain, whereas the remaining two samples yielded the tobacco strain. Broadbent (1962) established that dry smoking tobacco infected with the tobacco strain. of TMV is hardly infectious to tomato but may become very much so when it is wetted. Also he found the tobacco strain in grafted tomato plants, which according to my own experience cannot be regarded as pure coincidence. For during the act of grafting the young plants are handled very intensively and the chances of infection with the tobacco strain from contaminated hands or razors are greater than with any other treatment. It would seem therefore that the occurrence of the tobacco strain could be connected with the different ways of consuming tobacco and the practice of grafting tomatoes. Thus the tobacco strain may have become nearly extinct in tomato crops together with the habit of chewing tobacco instead of smoking it. Perhaps the role of the tobacco strain as a cause of tomato mosaic has been overemphasized compared with that of other strains. I am inclined to think that if a differential host was available for the yellow ringspot strain it would be found to be far more important than the tobacco strain.

The yellow ringspot strain should probably be regarded as a group of strains showing minor differences in the amount and severity of ringspotting rather than as one distinct strain. The appearance of yellowing symptoms in a tomato crop following a period of high temperatures may be easily mistaken for a temperature effect upon the symptom expression of the tomato strain. Only a small proportion of single-lesion isolates from such plants are of the yellow ringspot strain.

The only representative of the summer necrosis strain would probably have been included with the yellow ringspot strain if, by chance, it had been isolated from a plant in a heated glasshouse in winter under natural light conditions. Its odd behaviour in the controlled environment cabinets, previously referred to, suggests that it may be contaminated by a green mosaic strain which is suppressed by temperatures of approximately 21 °C. For similar reasons the winter necrosis strain could have been classified with the tomato strain. The separation of the necrosis strains into summer and winter is justified because of their consistent seasonal symptom pattern and also because of the different reactions they incite on other test plants. In this connection the only clean differentiating feature of the two yellow mosaic isolates is their inability to produce clearly visible ringspot symptoms on tomato and tobacco plants. The yellow spots on tomato fruits appear mostly angular in outline because of the presence of the tomato strain. This strain is regularly observed to replace the yellow mosaic strain sooner or later during the development of the infected host plant. This may possibly result from a back-mutation (Friedrich Freksa et al., 1946) or the inadequacy of the single lesion method of isolation.

The symptoms shown by the yellow, necrosis and enation strains may represent the potential variability of the tomato strain. So far, only six isolates have been shown to produce the characteristic fern leaf symptom throughout the season. Where persistent fern leaf symptoms and failure to set fruit on at least three trusses are discovered the enation strain may be involved. However, it is difficult to confirm the presence of this strain and the tomato strain is usually isolated. Fern leaf symptoms caused by the latter strain in winter time vary greatly with the growing conditions. Selman (1941) suggested that enations may result from variations in plant watering. There are no clear differences between strongly distorting isolates of the tomato strains and those of the enation strain and they can only be separated by observations throughout different seasons of the year.

The gradation in the severity of symptoms of isolates of TMV obtained from tomato suggest that a countless number of natural strains might exist ranging from symptomless strains to necrosis-inducing strains. If the principles of evolution apply to such a natural strain population green mosaic strains like the prevailing tomato strain would undoubtedly qualify as 'the fittest to survive in a struggle for existence'. The remainder of the strains including severely distorting and yellow mosaic strains may be considered to be more specialized and therefore are only able to compete in specific conditions.

3.5 Conclusion

On the basis of symptom expression TMV isolates from tomato crops in the Netherlands can be classified into eight more or less distinct strains. From a practical point of view, however, the strains which cause severe symptoms will be removed by the grower. This will reduce the chances of the dispersal of these strains.

4 Differences in host range among strains of tobacco mosaic virus in relation to resistance breeding in the Netherlands

4.1 Introduction

Considering the practical impossibility of TMV control in tomatoes by preventive measures it is logical to expect that breeding for resistance may be the ultimate solution of the problem. Doolittle (1954) in realizing that the reservoir of resistance known to exist in wild *Lycopersicon* species had been barely tapped stated that progress in this field of investigation was hampered by the constant problem of mutable pathogens. McRitchie & Alexander (1963) working in Ohio, U.S.A. were the first to prove the existence of different pathogenic strains of TMV. Alexander (1962) found Dutch isolates to be related but not identical to three out of the four Ohio strains. The Ohio Strain III was not found among the limited number of isolates studied in the Netherlands.

Breeding work in the Netherlands by commercial seed producers started from resistant breeding lines generously supplied by Alexander. Independently, the Institute for Horticultural Plant Breeding (I.V.T.) in Wageningen started a breeding programme intended to utilize the resistance of the *L. peruvianum* accession I.V.T. 62237 and of *Solanum pennellii*. The latter was obtained from Rick in California, U.S.A. (Szteyn, pers. commun.). However, when specific virus strains were found which attacked plants derived from this breeding programme (Rast, 1966; 1967c) Alexander's breeding lines derived from the *L. peruvianum* accession P.I. 128650 (Hogenboom, pers. commun.) were used. Faced with the difficulty that breeders in this country were not allowed to use the Ohio strains for testing Rast (1967b) investigated Dutch strains using clones of the *L. peruvianum* accession P.I. 128655 and also *S. pennellii* in addition to the differentials used by McRitchie & Alexander (1963). Three pathogenic groups of isolates were distinguished in this way (Rast, 1968a).

Meanwhile Pelham (1966) by his study of the literature on the sources of resistance promoted a better understanding of the relationships between the different genes used in breeding work. Furthermore he proposed a scheme for the identification of TMV strains based on the reaction of tomato hybrids carrying one or more known genes for resistance (Pelham, 1968). These included a gene for tolerance *Tm*-1 and both *Tm*-2 and *Tm*-2^a, which confer resistance based on hypersensitivity.

In this chapter the variability of TMV isolates as the main source of the trouble experienced during the attempts to arrive at a satisfactory strain classification is discussed. The adaptive changes observed with a number of isolates following passage through certain hosts will receive particular attention since they may lead to the

development of new pathogenic strains. This chapter will also give an account of the failure to connect differences in host range with differences in symptom expression on susceptible hosts.

4.2 Material and methods

4.2.1 Plant material

Most of the work on the differentiation of pathogenic strains of TMV has been done with clonal test plants which included the *L. peruvianum* accessions P.I. 126945, P.I. 128650, P.I. 128655 and I.V.T. 62237, *S. pennellii* and the *L. esculentum* breeding lines CStMW-18, Craigella *Tm*-1/+, Craigella *Tm*-2/+, Craigella *Tm*-2^a/+ and Craigella *Tm*-1/+. *Tm*-2/+. Test plants of the wild species *L. peruvianum* and *S. pennellii* were raised from single-node cuttings of about 3 to 5 cm length with part of the leaf attached. As this method did not work out satisfactorily for *L. esculentum* this species was propagated by using side shoots which were obtained by pruning back a number of source plants. Rooting of the cuttings was stimulated by the use of a powder formulation of β-indole acetic acid (Rhizopon A). The cuttings were raised individually in plastic multipots containing a mixture of two parts of normal potting soil, one part of sand, one part of peat and a small amount of lime. The pot size was varied according to the plant species; for the wild species there were 73 individual pots in each multipot plate, which measured 50 × 30 cm, whereas those used for *L. esculentum* had 51 pots. To prevent the rooting substrate from drying out the multipots were embedded in a 15 cm layer of well-moistened peat. A high relative humidity was maintained by placing a wooden framework with wire-netting over the freshly made cuttings and covering it with sheets of wetted filter paper and polythene. Direct wetting of the cuttings was avoided during the first two days to prevent the incidence of grey mould (*Botrytis cinerea*) which was further checked by dustings with TMTD. The polythene covering was removed after one week, while the sheets of filter paper were left for another week to shade the cuttings during sunny periods.
The plants grown from the cuttings were transplanted into 10-cm plastic pots after three weeks and inoculated after another week. As the use of these pots required too much labour, particularly for their disinfection, they were later on replaced by 3 – 1 plastic pots. These were used mainly for the wild species with a maximum of five individual plants grown together. The plants were well spaced to prevent contamination between groups of potted plants for at least two weeks following their inoculation.

4.2.2 Inoculum and inoculum techniques

Inocula for the tests were prepared preferably from fresh leaves from 'Samsun' tobacco plants which had been inoculated each with a single-lesion isolate one or two weeks previously. For each test approximately 1 g of leaves was ground with

water and diluted to give a volume of 40 ml. When fresh inocula were not available dried leaf material of single lesion isolates was treated similarly and used as inoculum. The inocula were then applied each to one 'Xanthi nc' and one 'Samsun' plant in addition to the clonal test plants. If inoculum prepared from dried material produced less than 10 lesions on the 'Xanthi nc' plant fresh inoculum was made from the 'Samsun' plant and the inoculation repeated. Following inoculation the remaining inoculum was not discarded but frozen and stored for at least the duration of the test. Similarly, leaf samples of all inoculated 'Samsun' plants were harvested dried for one or two days at 50°C and stored in paper bags. Later deep-frozen purified virus suspensions obtained following the column chromatographic method by Venekamp & Mosch (1964) were used for comparative purposes.

For mechanical inoculation the plants to be tested were first lightly dusted with carborundum, which was then swabbed from the leaves with a piece of cotton soaked in the inoculum. During inoculation the leaves were supported with a disk of filter paper. Because of increasing damage with length of use of the cotton swabs this technique was abandoned in later experiments and instead the leaves were rubbed gently between inoculum-wetted fingers. After inoculation remnants of carborundum were removed from the test plants by a thorough spray. Hands were washed twice with soap and water between inoculations of separate batches of test plants. Skimmed milk (Jaeger, 1966) was used as a dip for hands and tools for the pruning operations which were performed simultaneously with the reading of symptoms. Symptoms were recorded at least twice, the first time usually between two and three weeks after inoculation, the second time after three more weeks. When pruned back drastically the first time some of the lowermost inoculated leaves were left on the plants.

Only two or three of the developing new shoots were allowed to grow. A fertilizer solution was given to the plants every two weeks and aphids and spider mites were controlled when necessary. Tests were terminated with the assay of at least some of the plants which had remained symptomless or had shown a dubious reaction. Inoculated leaves were never used for assays.

4.2.3 Terminology

Because of the confusion in disease resistance terminology it will be necessary to give a definition of the terms used in this chapter. These definitions are adapted from the Dutch list (Anon., 1968) and Robinson (1969).

Pathogenicity will be used to denote the ability of TMV to cause disease in a host or range of hosts. Differences in pathogenicity between isolates of TMV may relate either to extent of host range or to severity of symptoms and may be described in terms of *aggressiveness* or *virulence*. An isolate causing green mosaic symptoms on a certain host range is more aggressive than another isolate causing yellow mosaic symptoms on a smaller number of plants of the same host range. The former isolate, however, is less virulent than the latter. In this chapter the term pathogenicity or its derivatives will be used with the emphasis on the aggressiveness rather than the

virulence of the isolates investigated.

Resistance is used to denote the ability of a host to hinder the establishment and further development of an infection by TMV to the extent that following inoculation it remains almost free from the virus. By contrast *susceptibility* referes to a host which may be easily infected by TMV and which allows rapid multiplication. According to the symptoms produced or as a result of an assay a susceptible host may be called either *sensitive* or *tolerant*. For convenience the development of symptoms will be considered implicit to susceptibility which in the absence of symptoms will be separately referred to as tolerance.

Hypersensitivity should be mentioned here as a mechanism for the resistance found in *L. peruvianum* although it does not result in the rapid localization of TMV in necrotic lesions. In breeding for resistance to TMV hypersensitivity is mostly used to denote a top necrosis followed by a permanent stunting of growth. This reaction may be observed when a resistant *L. peruvianum* is grafted on to a susceptible rootstock (Alexander, 1962). It very frequently occurs following mechanical inoculation of tomato breeding lines heterozygous to either *Tm*-2 or *Tm*-2^a. Usually the appearance of systemic necrosis on top leaves of *L. peruvianum* is only the initial phase and is followed by the development of mosaic symptoms in further growth. The plant showing this reaction is recorded as susceptible. However, a plant showing top necrosis without mosaic symptoms should be considered resistant as sub-inoculation to another plant of the same clone will normally fail to produce any effect. It should be noted that absolute resistance or *immunity* does not exist in *L. peruvianum*.

4.3 Variability of TMV isolates on clonal test plants

Because former attempts to classify TMV isolates on seedlings of the differential hosts of Alexander (1962) produced erratic results I switched to using vegetatively propagated plants. By fixing the distribution pattern of genes for resistance in the host population it was expected that this would enable a reliable comparison between TMV isolates.

4.3.1 The effect of climatic factors

Starting with a differential host range consisting of 30 clones of *L. peruvianum* P.I. 128655 (Rast, 1967 b) the reactions observed with a number of isolates still varied between tests. This is shown by some of the results obtained with six isolates in Table 5. Considering that the two inocula for each test for one specific isolate were derived from exactly the same sample of dried leaves, the differences in host reaction between tests are difficult to explain. It is possible that the susceptibility of a host as well as symptom expression are subject to seasonal variation. In this connection it should be noted that the isolates denoted with A.8, SL^c and MH (Table 5) were first tested in the autumn of 1965 and again in the spring of 1966.

At the time that these tests were done it was known that high temperatures could

Table 5. Differences in resistance/susceptibility of 30 clones of *Lycopersi con peruvianum* P.I. 128655 to 6 isolates of TMV in 2 tests, 1 and 2. Each sign +, − or * represents 1 plant.

Clone no.	Isolate											
	SPS		ENP		A.8		SL[a]		SL[c]		MH	
	1	2	1	2	1	2	1	2	1	2	1	2
1, 5	−[1]	−	−	−	−	−	−	−	−	−	−	+
2	−	+[2]	−	−	−	+	−	−	−	+	+	+
3, 4, 9, 10, 21	−	−	−	−	−	−	−	−	−	+	−	+
6, 20, 23, 25, 28	+	+	+	+	+	+	+	+	+	+	+	+
7	−	−	−	−	+	+	−	+	−	−	−	−
8, 15, 26, 27	−	−	−	−	−	−	−	−	−	−	−	−
11	−	−	−	−	−	−	−	+	−	+	+	+
12	−	−	−	−	−	+	−	−	−	+	−	+
13	−	−	−	−	−	+	−	+	−	+	−	+
14, 24	−	−	−	−	+	−	−	−	−	+	−	+
16	−	−	−	+	+	−	+	+	−	+	−	−
17	−	+	−	−	+	+	+	+	−	+	−	+
18	−	−	−	−	+	−	−	−	*[3]	−	−	−
19	−	−	+	−	−	−	−	−	−	+	+	+
22	−	−	−	−	−	−	−	+	*	+	−	+
29	−	−	−	−	+	+	−	−	−	+	−	+
30	−	−	−	−	+	−	−	+	−	−	−	−

1. − = resistant (inoculated plants did not show symptoms and did not show symptoms and did not contain virus).
2. + = susceptible (inoculated plants showed symptoms).
3. * = tolerant (inoculated plants did not show symptoms but contained virus).

accelerate the appearance of a systemic necrosis on resistant tomato hybrids (Hogenboom, pers. commun.; Cirulli & Alexander, 1969). To investigate whether a similar relationship would apply to *L. peruvianum* P.I. 128655 three batches of plants were exposed to a higher temperature than was normally maintained for the routine tests. From the results presented in Table 6 it may be concluded that high temperatures will not necessarily cause an increase in the number of plants producing symptoms. While this may be true for the isolate SL[a] the results with A.8 would rather suggest the opposite tendency viz. that high temperatures may also induce otherwise susceptible plants to become resistant.

In another test performed in controlled environment cabinets plants of the *L. peruvianum* clone I.V.T. 62237 – 5 were kept following inoculation for three weeks at a nearly constant temperature of 22 or 27°C. Out of 10 isolates, each inoculated to batches of two or three plants for each temperature treatment, only MH caused mosaic symptoms. These appeared on two of three plants at 22°C and on all three plants at 27°C. These results in confirming those obtained under normal glasshouse conditions

Table 6. Differences in resistance/susceptibility of 14 clones of *Lycopersicon peruvianum* P.I. 128655 to 3 isolates of TMV at normal (16 – 24°C, average 21°C) and high (24 – 34°C, average 27°C) temperatures. Each sign + or – represents 1 plant.

Clone no.	Isolate					
	SPS		A.8		SL[a]	
	normal	high	normal	high	normal	high
1, 5, 21, 26, 27	–[1]	–	–	–	–	–
7	–	+[2]	+	–	+	–
12	–	–	+	–	–	–
13	–	–	+	+	+	+
15, 19	–	–	–	–	–	+
16	–	–	–	–	+	+
18	–	–	–	+	–	+
22	–	–	–	–	+	–
29	–	–	+	–	–	+

1. – = resistant (inoculated plants did not show symptoms and did not contain virus).
2. + = susceptible (inoculated plants showed symptoms).

suggest that the visible reactions observed had less to do with high temperatures than with a matching host-virus-combination. Since possible temperature effects were not clearly indicated they are unlikely to account for the irregularities observed in the reaction of *L. peruvianum* plants.

4.3.2 Effects of host passage

In a later stage of the investigation the 30 clones of *L. peruvianum* P.I. 128655 were replaced by another differential host range which consisted of the *L. peruvianum* accessions P.I. 128655, P.I. 126945, P.I. 128650, the *L. esculentum* selection CStMW–18 and *S. pennellii*, each of them represented by five clones. As one of the new clones of P.I. 128655 was susceptible to all isolates tested so far the clone no. 27 of the previous series was used to replace it as clone no. 8 of the new series. The new differential host range was completed with the *L. peruvianum* clone I.V.T. 62237–5.

Observations made soon after starting work with the new differential host range indicated that changes in pathogenicity may occur as a result of host passage. This possibility was first suggested by the increase in aggressiveness of the isolate GeRo following its reisolation from a susceptible plant (Table 7) and confirmed with the isolate SK–68–2. Again the isolates derived from susceptible differentials were more aggressive than the original isolate. However, the differences between the three isolates tested in June were slight when compared with the original isolate tested in April 1969. Even the original isolate stored in deep-freeze gave a quite different reaction in the

Table 7. Changes in pathogenicity shown by isolates of TMV on clonal test plants of *Lycopersicon peruvianum* following reisolation from susceptible plants. Each sign +, − or * represents 1 plant.

Isolate	Date of test	Plant														
		I.V.T. no.	P.I.126945					P.I.128655					P.I.128650				
		62237 – 5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
GeRo																	
Original isolate	May '68	.	−[1]		+[2]	−	−	+	−	.	+	−	+	−	−	−	−
Reisolated from 11	Aug. '68	−	+	+	+	+	+	+	−	.	+	+	+	−	−	−	−
SK–68–2																	
Original isolate	Apr. '69	−	−	−	−	−	−	−	−	.	+	.	+	−	−	−	−
Original isolate	June '69	+	+	+	+	−	−	+	−	−	.	+	+	−	−	−	−
Reisolated from 9	June '69	+	+	+	+	+	−	+	−	−	.	+	+	−	−	−	−
Reisolated from 1	June '69	+	+	+	+	+	+	+	−	−	.	+	+	−	−	−	−
SG–64/1																	
Original isolate	July '70	−	−	*[3]	−	−	−	+	−	−	−	−	−	−	−	−	−
Reisolated from 6	June '71	+	+	+	+	+	+	.	−	−	+	+	+	−	−	−	−
Original isolate	May '70	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−
Reisolated from 7	June '71	−	−	+	+	+	−	.	−	−	+	+	+	−	−	−	−
SG–64/2																	
Original isolate	June '70	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Reisolated from 8	June '71	−	−	−	−	−	−	.	−	−	−	−	−	−	−	−	−
SG–64/3																	
Original isolate	May '70	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−
Reisolated from 9	June '71	+	+	+	+	+	+	.	−	−	+	+	+	−	−	−	−

1. − = not infected.
2. + = infected with symptoms.
3. * = infected without symptoms.

second test. Therefore, the increases in aggressiveness observed with both GeRo and SK–68–2 could not be explained as a result of host passage only.

The effect of host passage on pathogenicity was further investigated with TMV sample SG–64 from which 20 single-lesion isolates were prepared. The results obtained with three single-lesion isolates of SG–64 designated SG–64/1, SG–64/2 and SG–64/3 are presented in Table 7. The isolates from plants no. 6 and 9 had a similar host range and were more aggressive than the isolate from plant no. 7. All three had definitely gained in aggressiveness compared with their respective parent isolates, but the isolate from plant no. 8 became less aggressive. It should be noted that the initial mosaic symptoms in plants no. 7 and 8 faded with time and repeated clippings. TMV was recovered from these plants by inoculation to *N. clevelandii* but not after inoculation to 'Xanthi nc'.

Table 8. Changes in pathogenicity shown by isolates of TMV on 5 seedlings of *Lycopersicon esculentum* cv. 'Avires' following reisolation from susceptible plants of *L. peruvianum*. For plant numbers see Table 7. Each sign + or − represents 1 plant.

SG–64/1[1]					
Original isolate	+[2]	+	+	+	+
Isolate from plant no. 6	+	−[3]	−	−	−
SG–64/2[1]					
Original isolate	+	+	+	+	−
Isolate from plant no. 8	+	−	−	−	−
SG–64/3[1]					
Original isolate	+	+	+	+	+
Isolate from plant no. 9	+	+	−	−	−
SG–64/4					
Original isolate	+	+	+	+	+
Isolate from plant no. 6	+	−	−	−	−

1. The same isolates as listed in Table 7.
2. + = symptoms.
3. − = no symptoms.

Clearly the isolates GeRo and SK–68–2 were pathogenic only to *L. peruvianum* plants. The original isolates of SG–64, however, were also capable of infecting plants of *S. pennellii* and *L. esculentum* CStMW–18. Unfortunately these host species were not available at the time that isolates of SG–64 had been obtained from susceptible plants of *L. peruvianum*. Instead the isolates were tested on seedlings of the tomato hybrid 'Avires' carrying the *Tm*–1 gene for tolerance. From the reactions shown in Table 8 it is obvious that their virulence towards cv. 'Avires' had decreased following passage through susceptible *L. peruvianum* plants.

It sometimes occurred that the assay of resistant *L. peruvianum* plants on 'Xanthi nc' yielded one or two lesions. A number of the isolates derived from such lesions tended to show an increased aggressiveness towards *S. pennellii* and *L. esculentum* CStMW–18 when compared with the original isolates. The tests with the isolates of SK–68–2 (Table 9) were carried out simultaneously with those appearing in Table 7. The isolate SK–68–2 after being recovered from a resistant *L. peruvianum* plant retained only part of its former aggressiveness towards this species. Contrary to the isolates listed in Table 7 it caused mosaic symptoms in plant no. 1 only. The other isolates of Table 9 viz. GU–68, GK–68 and GH–68 were never observed to infect *L. peruvianum*.

A similar shift in aggressiveness was shown by the isolate MH after being passed twice through *Cyphomandra betacea*. Each time MH could only be recovered from the inoculated leaves of this solanaceous host which is reported to be resistant to TMV

Table 9. Changes in pathogenicity shown by isolates of TMV on clonal test plants of *Solanum pennellii* and *Lycopersicon esculentum* CStMW–18 following reisolation from lesions produced by assay of resistant plants of *L. peruvianum*. Each sign represents 1 plant.

Isolate	Date of test	*S. pennellii*			*L. esculentum*			
		16	17	20	21	23	24	25
SK–68–2[1]								
Original isolate	Apr. '69	−[2]	−	−	−	.	.	.
Original isolate	June '69	−	−	−	−	.	−	−
Reisolated from *L. peruvianum* plant 12[3]	June '69	+[4]	+	+	*[5]	*	*	*
GU–68								
Original isolate	Apr. '69	−	−	−	−	.	.	.
Reisolated from *L. peruvianum* plant 2	July '69	+	+	+	*	*	*	*
GK–68								
Original isolate	Apr. '69	−	−	−	−	.	.	.
Reisolated from *L. peruvianum* plant 13	July '69	+	+	+	*	*	+	+
GH–68								
Original isolate	Apr. '69	−	−	−	−	.	.	.
Reisolated from *L. peruvianum* plant 3	July '69	+	+	+	+	+	*	*

1. The same isolates as listed in Table 7.
2. − = not infected.
3. For plant numbers see Table 7.
4. + = infected with symptoms.
5. * = infected without symptoms.

(Smith, 1959). Following the repeated passage MH was no longer pathogenic to *L. peruvianum* (see Table 5 for comparison) but caused mosaic symptoms on two out of five plants of *S. pennellii* and on four out of five plants of *L. esculentum* CStMW–18. This reaction had not previously been observed.

Sometimes the appearance of mosaic on a presumed resistant plant was confined to one of its branches. Assays confirmed that the affected branch contained virus whereas the others did not. Repeated tests either with the original inoculum or with inoculum prepared from the affected parts of the plant failed to reproduce mosaic symptoms on fresh plants of the same clone. The observation that free movement of the virus was obstructed in the infected plant (Robb, 1964) and that it was not transmissible to genetically identical plants suggest the presence of some unknown factor for resistance. In discussing similar observations Alexander (1962) suggested that one such factor may be responsible for partially inhibiting virus multiplication and for preventing transmission of the virus. It is possible that the occurrence of the symptoms previously mentioned for the isolates of SG–64 on plants no. 7 and 8 may be explained in a similar manner.

Unexpected changes in the pathogenicity of the virus isolate GM–65 occurred with increasing duration of an established infection in a susceptible host (Table 10). This

Table 10. Changes in pathogenicity shown by the isolate GM–65 on clonal test plants of *Lycopersicon peruvianum*. Each sign +, – or * represents 1 plant.

Inoculum used	Date of test	I.V.T. 62237–5	P.I. 126945					P.I. 128655					P.I. 128650				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Prepared from 'Samsun' following inoculation with original isolate	April '69	–[1]	–	–	–	–	–	+[2]	–	–	–	–	–	–	–	–	–
Same inoculum as used on April '69, taken from deep-freeze	June '69	–	–	–	–	–	–	–	–	–	.	–	–	–	–	–	–
Prepared from *L. peruvianum* plant no. 6	June '69	–	–	–	–	–	–	–	–	–	.	–	–	–	–	+	–
Same inoculum as used on April '69, taken from deep-freeze	Sept. '69	–	+	–	–	–	–	+	–	–	.	–	–	–	–	–	–
Same inoculum from *L. peruvianum* plant no. 6 as used in June '69, taken from deep-freeze	Sept. '69	+	+	–	–	+	–	–	–	+	.	+	–	–	–	–	–
Prepared from *L. peruvianum* plant no. 14 infected in test of June '69	Sept. '69	–	–	–	–	+	–	–	–	–	.	–	–	–	–	–	–
Prepared from *L. peruvianum* plant no. 14 infected in test of June '69	June '71	–	–	–	–	–	–	.	+	–	–	+	–	–	+	+	.
Prepared from *L. peruvianum* plant no. 14 infected in test of June '71	July '71	–	–	–	–	–	–	–	–	–	–	.	–	–	–	–	–
Prepared from *L. peruvianum* plant no. 13 infected in test of June '71	July '71	–	–	–	–	–	–	–	+	–	–	.	–	*[3]	+	+	+
Prepared from *L. peruvianum* plants nos. 13, 14 or 15 infected in test of July '71	Sept. '71	–	.	–	.	.	.	–	+	+	.	–	–	+	+	+	+
The host range of the isolate GeRo added for comparison		+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–

1. – = not infected.
2. + = infected with symptoms.
3. * = infected without symptoms.

isolate initially caused mosaic symptoms on *L. peruvianum* plant no. 6 and from previous experience it was expected that passage through this host might result in a similar increase in aggressiveness shown by other isolates (Table 7). Tests in June 1969 (Table 10) with the reisolated virus caused a fully systemic infection of the hitherto resistant plant no. 14. Further tests in September 1969 showed the expected increase in aggressiveness as five plants of the differential series developed mosaic. However, virus from the visibly infected plant no. 6 repeatedly failed to reinfect plants of the same clone. Meanwhile the attempts to induce mosaic symptoms on fresh plants of the *L. peruvianum* clone no. 14 also remained unsuccessful. This situation lasted for about two years when finally mosaic symptoms were produced on four plants. Subsequent tests showed that an isolate from a recently infected differential no. 14 was markedly less aggressive. By contrast the repeated passage through *L. peruvianum* no. 13 apparently exerted a stabilizing influence upon host range. The isolates of GM–65 from plants no. 13, 14 or 15 had become pathogenic on a host range resistant to infection by the isolates listed in Table 7.

4.3.3 The effect of different methods of storage

By chance I found that different methods of storage of some isolates affects pathogenicity. Normally isolates were kept in dried infected leaves but a number of selected isolates were purified and stored in a deep-freeze. In a final experiment 30 characterized isolates were compared on a full host range. For each isolate a dried leaf sample was used to inoculate one 'Samsun' plant which in turn provided fresh inoculum for the test. Five isolates including the Ohio strain III which were expected to cause mosaic symptoms on CStMW–18 and *S. pennellii* behaved accordingly. However, eight out of nine other isolates including the Ohio strain IV which should have caused a reaction on the *L. peruvianum* plants failed to do so. The only isolate which behaved as expected had been kept in dried leaves for less than two years. The experiment was then repeated with fresh inocula prepared from plants of a universally susceptible *L. peruvianum* clone but the results remained unsatisfactory.

Subsequently a limited number of *L. peruvianum* clones were tested using six out of the nine isolates from deep-frozen inoculum[1]. Isolates MH and Ohio IV behaved exactly alike and appeared to have retained their aggressiveness in deep-freeze as did two other isolates omitted from Table 11. The isolate VW had lost part of its former aggressiveness but the inoculum was prepared from a source plant which may also have contributed to the decrease in aggressiveness. However, the isolate caused mosaic symptoms on the *L. peruvianum* plant no. 10 and it is possible that passage through this plant may eventually completely restore its aggressiveness.

1. Every time a batch of tomato plants was inoculated for chromatographic purification the inoculum used was also applied to a 'Samsun' tobacco plant of which a leaf sample was dried and stored afterwards.

Table 11. Differences in pathogenicity shown by isolates of TMV on clonal test plants of *Lycopersicon peruvianum* as a result of different methods of storage. Each sign + or − represents 1 plant.

Isolate	Method of storage	Inoculum	Date of test	I.V.T. 62237–5	P.I. 126945 2	P.I. 128655 7	P.I. 128655 8	P.I. 128655 10	P.I. 128650 11
Ohio IV	dried leaves	Freshly prepared from 'Samsun' and a generally susceptible *L. peruvianum*[1]	May '71 and July '71	−[2]	−	−	−	−	−
	deep-freeze	purified suspension diluted 1:100	Sept '71	+[3]	+	−	−	+	+
MH, from I.V.T. 62237–5	dried leaves	freshly, prepared from 'Samsun' and a generally susceptible *L. peruvianum*[1]	May '71 and July '71	−	−	−	−	−	−
	deep-freeze	purified suspension diluted 1:100	Sept. '71	+	+	−	−	+	+
GeRo, from P.I. 128650 no. 11	dried leaves	freshly prepared from 'Samsun' and a generally susceptible *L. peruvianum*[1]	May '71 and July '71	−	−	−	−	−	−
	deep-freeze	purified suspension diluted 1:100	Sept. '71	+	+	−	−	+	+
VW, from P.I. 128655 no. 8	dried leaves	freshly prepared from 'Samsun'	May '71	−	−	−	−	−	−
		freshly prepared from generally susceptible *L. peruvianum*	July '71	−	−	−	−	+	−
		freshly prepared from plant no. 10	Sept. '71	+	+	−	+	+	+
	deep-freeze	purified suspension diluted 1:100	Sept. '71	−	−	−	−	+	−

1. The two successive tests with the freshly prepared inocula were carried out on a complete series of *L. peruvianum* clones and gave negative results.
2. − = not infected.
3. + = infected with symptoms.

4.4 The pathogenicity of yellow strains of TMV

In an earlier paper Rast (1967b) hinted at the possible significance of yellow strains of TMV for resistance breeding. Apart from the convenience of their striking symptoms for testing purposes Messiaen & Maison, 1962) they were apparently more aggressive than strains which cause green mosaic symptoms. Isolates of the yellow ringspot strain were involved with the breakdown of the resistance found in the *L. peruvianum* accession I.V.T. 62237 (Rast, 1967c) and were among the first to be associated with the Ohio strain III (Rast, 1968a). Isolate GM-65 discussed in the previous section was also a yellow ringspot strain. Of the isolates derived from the TMV sample SL the one showing a green mosaic (SL^a) was less aggressive than the one showing a yellow mosaic (SL^c). See Table 5.

A search for similar relationships among other isolates was made using 17 dried-leaf samples collected in the Netherlands by Alexander (1962). The isolation of the prevalent tomato strain and an attendant yellow strain from each of these samples yielded 11 comparable pairs of isolates. The results of subsequent tests on clonal test plants of *L. peruvianum* and on seedlings of the tolerant cv. 'Avires' showed little difference in aggressiveness, either between the isolates obtained from one sample or between the pairs of isolates. Only two isolates caused mosaic symptoms on plants of the *L. peruvianum* clone no. 9 and these symptoms were a green and not a yellow mosaic. When reisolated and tested again they showed a further increase in aggressiveness. There are no apparent reasons for the assumption that yellow strains are more aggressive than the green mosaic strains from which they derived and the involvement of the yellow ringspot strain referred to previously was probably coincidental.

4.5 The classification of pathogenic strains of TMV

The variability in reaction of clonal test plants to TMV isolates hardly allows for a rigid classification into strains. At best the isolates which cause similar reactions may be grouped according to their possible relation to the Ohio strains described by Alexander (1962) and McRitchie & Alexander (1963). The 115 isolates obtained from susceptible tomato crops and examined during a period of about four years can be divided into four groups. As the distinction between the Ohio strains I and II is of little interest to plant breeders the isolates related to these strains have been placed in one group. Two further groups correspond with Ohio strains III and IV whereas the fourth group has not previously been described. A characteristic of the four pathogenically different groups of TMV isolates based on the behaviour of the clonal test plants is given in Table 12.

Group 1 A number of isolates (21) in this group was only pathogenic on universally susceptible plants. Others were found to infect the differential hosts, *S. pennellii* and the *L. esculentum* breeding line CStMW-18, either with (28) or without (45) symptoms. The reactions were inconsistent and never involved all of the plants used. The

Table 12. Preliminary classification of TMV isolates into 4 pathogenically different groups on the basis of resistance or susceptibility of the clonal test plants.

Differential host	Number of clones tested	Group of TMV isolates			
		1	2	3	4
Solanum pennellii	5				
Lycopersicon esculentum		V[1]	S[2]	R[3]	R
CStMW-18	5				
Lycopersicon peruvianum					
P.I.126945	5				
I.V.T.62237-5	1	R	R	S	R
P.I.128650	1				
P.I.128655	3				
P.I.128655	2				
P.I.128650	4	R	R	R	S

1. V = variable behaviour of clonal test plants. A batch may be either resistant as a whole or may consist of a mixture of both resistant and susceptible individuals, of which the latter may or may not react visibly of infection.
2. S = susceptible; plants show symptoms.
3. R = resistant; plants do not show symptoms and do not contain virus.

isolates which caused typical symptoms on some of the plants represented mixtures of strains which might have been separated by further single lesion isolation. The Ohio strains I and II of McRitchie & Alexander (1963) come into Group 1. Their differentiation was based upon the reactions of CStMW-18, which proved resistant to the former strain but behaved as a symptomless carrier to the latter strain.

Group 2 The 7 isolates of this group visibly infected all plants of *S. penellii* and CStMW-18. Isolates which developed this capacity as the result of a passage through resistant plants of *L. peruvianum* were not included. The isolates of Group 2 are closely related to the Ohio strain III in the reaction of CStMW-18. According to McRitchie & Alexander (1963) this strain should cause a similar reaction on *L. peruvianum* P.I. 126945. This, however, could not be verified neither with the isolates of Group 2 nor with an isolate of Ohio strain III obtained from Alexander. Probably the selection of P.I. 126945 used in this work was different from the one used by the authors referred to.

Group 3 The 12 isolates in this group produced symptoms on a number of *L. peruvianum* plants. The normal host range included plants of the clone I.V.T. 62237-5, three clones of P.I. 128655, all five clones of P.I. 126945 and one clone of P.I. 128650.

Plants of the two remaining clones of P.I. 128655 were inconsistently infected by different isolates of this group. The similarity in host range shown by the isolates of Group 3 and the Ohio strain IV on plants of *L. peruvianum* suggests they are closely related. According to Alexander (1962) and McRitchie & Alexander (1963) the latter strain should also cause a uniform symptomless infection of CStMW–18. Isolates of Group 3 differ from Ohio IV in that they never infected plants of CStMW–18. Some may have lost their infectivity towards this host following passage through susceptible plants of *L. peruvianum* as did SG–64 (see Table 8).

Strains similar, if not identical, to the Ohio strains may be found among the isolates of the three groups discussed so far.

Group 4 Isolate GM–65 infected only those *L. peruvianum* plants not normally infected by the isolates of the previous group. This isolate originated in experimental conditions and the chances of finding its natural counterparts seemed very remote. Recently, however, such an isolate was obtained at a breeder's holding from plants showing a mosaic symptom and presumed to be resistant. In a subsequent test this isolate, MR–72, infected tomato breeding lines homozygous to $Tm\text{–}2^a$, the gene for resistance known to be derived from *L. peruvianum* P.I. 128650. The two isolates constituting the fourth group have not previously been recorded in the literature and represent an entirely new strain.

4.6 An evaluation of Pelham's system for strain classification

The system of strain classification developed by Pelham (1968) is based on the concept of a gene-for-gene relationship. The differential hosts consist of tomato hybrids with either one or a combination of any of the three known genes for resistance. The strains are given a numerical notation which corresponds with the genotype of the differential host reacting visibly to infection. So, the Ohio strains I and II which both cause typical symptoms only on the universally susceptible host fall into Strain O. Similarly, the Ohio strains which visibly infect the differential hosts carrying the genes *Tm*–1 and *Tm*–2 respectively have to be named Strains 1 and 2 respectively. An obvious advantage of Pelham's system is that the tomato hybrids may be used as seedlings in the cotyledon stage. This is impracticable with the delicate seedlings of the wild species which furthermore represent totally unknown genotypes. Pelham's system therefore deserves general acceptance by all those concerned with breeding for resistance to TMV in tomatoes.

For a first trial in this investigation 45 isolates belonging to the four previously discussed groups were tested on cuttings made from Pelham's differentials. For a comparison the results obtained with 23 isolates including the Ohio strains are presented in Tables 13 and 14. The remaining 22 isolates will be discussed afterwards.

It should be noted that the reactions for the *L. peruvianum* clones in Table 13 have been compiled from several tests. The reactions of *S. pennellii* and *L. esculentum*

Table 13. Resistance/susceptibility of clonal test plants of *Solanum pennellii*, *Lycopersicon esculentum* CStMW–18 and the *L. peruvianum* accessions I.V.T. 62237, P.I. 126945, P.I. 128655 and P.I. 128650 to 23 isolates of TMV. Each sign + – or * represents 1 plant.

Isolate	Pathogenic group (this work)	*S. pennellii*					*L. esculentum* CStMW–18					*L. peruvianum*														
		16	17	18	19	20	21	22	23	24	25	I.V.T. 62237–5	P.I. 126945 1	2	3	4	5	P.I. 128655 6	7	8	9	10	P.I. 128650 11	12	13	14	15
MA	1	–	–	–	*[2]	–	–	–	*	–	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SD	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ENP	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GdK	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GPga	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MKv	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SBK	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SPS	2	+[3]	+	+	+	+	+	+	*	+	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NH–69	2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SO–69	2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GeB1	2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GB–68–2	2	+	+	+	+	+	+	*	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SJ–64	3	–	–	–	–	–	–	–	–	*	–	–	–	–	+	–	–	+	–	–	+	+	–	–	–	–	–
MH	3	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–
VW	3	–	–	–	–	–	–	–	–	–	*	+	+	+	+	+	+	+	–	+	+	+	+	–	–	–	–
GeRo	3	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–
SK–68–2	3	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–
SG–64	3	–	–	*	–	–	*	–	*	–	–	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–
GM–65	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	+	+
Ohio–I	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ohio–II	1	–	*	–	–	–	–	–	*	*	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ohio–III	2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ohio–IV	3	*	–	–	–	–	–	–	*	*	–	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–

1. – = resistant. 2. + = susceptible. 3. * = tolerant.

Table 14. Resistance/susceptibility of clonal test plants of the tomato cultivar Craigella with genotypes *Tm*-1/+, *Tm*-2/+, *Tm*-1/+ · *Tm*-2/+ and *Tm*-2^a/+ to 23 isolates of TMV. Each sign +, − or * represents 1 plant.

Isolate	Pathogenic strain (Pelham, 1968)	Symptoms of susceptible host plants	*Tm*-1/+					*Tm*-2/+					*Tm*-1/+ · *Tm*-2/+					*Tm*-2^a/+				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
MA	0	green mosaic	+[1]	−[2]	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SD	0	green mosaic	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ENP	0	leaf distortion	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GdK	0	yellow ringspotting	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GPga	0	yellow mosaic	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
MKv	0	leaf vein necrosis	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SBK	1	severe necrosis	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SPS	1	green mosaic	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
NH–69	1?	leaf distortion	+	+	*[3]	+	+	−	−	−	−	○[4]	●[5]	●	−	●	●	●	●	−	●	●
SO–69	1	yellow mosaic	+	+	+	*	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GeB1	1	yellow mosaic	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GB–68–2	1	yellow ringspotting	+	+	+	*	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SJ–64	1 + 2?	green mosaic	+	*	+	+	+	●	●	●	●	●	−	−	−	−	−	−	−	−	−	−
MH	2	yellow ringspotting	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−
VW	2	yellow ringspotting	*	−	−	−	−	+	+	+	+	+	−	−	−	−	−	+	−	−	*	−
GeRo	1 + 2?	yellow ringspotting	+	*	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
SK–68–2	2	yellow ringspotting	−	*	*	−	−	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−
SG–64	2	yellow ringspotting	*	−	−	−	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−
GM–65	2^a	yellow ringspotting	−	−	−	−	−	−	○	−	○	−	−	−	−	−	−	+	+	+	+	+
Ohio–I	0	green mosaic	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	●	−	○
Ohio–II	0	green mosaic	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	●	−	○	○	○
Ohio–III	1	green mosaic	+	+	+	+	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ohio–IV	2	green mosaic	+	−	+	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−

1. + = susceptibility.
2. − = resistance or tolerance particularly suspected to occur with Craigella *Tm*-1/+.
3. * = tolerance confirmed by assay.
4. ○ = mild necrotic reaction.
5. ● = severe necrotic reaction.

CStMW–18 are derived from one final experiment. Except for the isolates SPS and GB–68–2 the isolates of Group 2 produced symptoms on all plants of CStMW–18. The isolate SJ–64 is probably more aggressive than is suggested by the small number of positive reactions on *L. peruvianum* plants. In previous work (Rast, 1967b) the same isolate appeared to be as aggressive as the isolate GeRo. These isolates, then called numbers 4 and 10 respectively, were capable of infecting 12 and 10 plants respectively out of 30 plants of P.I. 128655. In a later experiment each of them infected 4 out of 11 *L. peruvianum* plants. The positive reactions with GeRo and other isolates of group 3 were further investigated as reported in Table 7.

With Pelham's isogenic differentials it was found that the symptoms of Craigella *Tm*–1/+ in particular were difficult to read. Nevertheless, assays were made only when a mild mottling of the plants suggested the presence of virus or when this could be reasonably expected. The latter situation occurred when in a similarly treated batch of plants one remained symptomless while the others showed typical symptoms. Negative signs in Table 14 may therefore stand for either resistance or tolerance that was not confirmed by assay. Positive signs were reserved for symptoms typical of the isolates concerned. In this connection it was useful to use isolates with characteristic symptoms which differed from a normal green mosaic.

As expected from their close relationship to the Ohio strains the four groups of isolates fitted well into Pelham's scheme. The isolates of Group 1 fell into Strain 0 except for SBK which, together with the isolates of Group 2, was characteristic of Strain 1. Similarly, four isolates of Group 3 were clearly identical to Strain 2 apart from an occasional positive reaction on Craigella *Tm*–2^a/+. The reaction of SJ–64 on Craigella *Tm*–2 makes it a typical of Strain 1. The necrotic reaction, contrary to similar initial reactions with other isolates e.g. NH–69, rapidly progressed and killed all the *Tm*–2 differentials. To explain for this violent reaction it is assumed that the isolate consisted of a mixture of Strains 1 and 2, Strain 1 inciting necrosis on the way while being carried along by the fully invasive Strain 2. The isolate GM–65 which caused mosaic symptoms only with Craigella *Tm*–2^a should be accordingly classified as Strain 2^a. Its position is not certain since in a preliminary test on seedlings similar symptoms were observed on a number of Craigella *Tm*–2/+ plants.

Pelham's tomato differentials enabled a finer distinction to be made between the isolates of Groups 1 and 2. The results with 17 isolates on Craigella *Tm*–1/+ showed that 11 out of 15 from Group 1 were classified as Strain 0, the others as Strain 1. One of two isolates from Group 2 conformed with Strain 1, whereas the other gave a typical Strain 0 reaction. In general, Craigella *Tm*–1/+ was more susceptible than corresponding hosts we used as seen by the results with the Ohio strains shown in Tables 13 and 14.

The necrotic reactions shown by the other differential hosts to some of the isolates were rather confusing. They prevented certain identification of the five remaining isolates that were not listed in the tables although four of them behaved similarly to isolate SJ–64 and were probably related to Strain 2. With the final isolate which was

a tobacco strain three Craigella *Tm*-2^a/+ plants reacted with mosaic symptoms, another with severe necrosis and the last plant remained symptomless. This suggests the existence of Strain 2^a long before resistant tomato breeding lines with the matching gene *Tm*-2^a were being released for commercial use.

4.7 Discussion

The variability of TMV has practical consequences both for testing procedures in relation to breeding for resistance and when considering the fate of resistant tomato cultivars after their release into commerce.

It has been shown that the pathogenicity of any given isolate may vary between tests probably as a result of seasonal influences. Also temperature may affect one isolate more than another. This suggests that tests should be repeated at different times of the year and, if possible, at both moderate and high temperatures. However, much of the variation found may be due to the fact that the isolates represent strain mixtures. This raises the question of the usefulness of the single-lesion technique as a method for biological purification of strains. The results with the isolate SG–64 suggest that there is apparently no advantage in applying this method. Although originally containing both Strains 1 and 2 the isolate yielded its Strain–2 component only following a long sustained culture in susceptible plants of *L. peruvianum*. The other isolates of Strain 2 were obtained without much difficulty after a single passage through *L. peruvianum*. Work by Pelham et al. (1970) suggests that it must be relatively easy to obtain Strain–1 isolates by host passage. The 'selection pressure' (Robinson, 1969) exerted by host passage might well be regularly exploited in the search for pathogenic strains. This should not be considered less natural than a serial transfer of single lesions from *N. glutinosa* or similarly reacting test plants.

Although the results obtained with Pelham's tomato hybrids for the identification of pathogenic strains were generally satisfactory some problems remain.

Firstly Craigella *Tm*–1/+ enabled the separation of isolates with *Strain*–1 characteristics from the Group–1 isolates we used but should the remainder of this group be indiscriminately classified as Strain 0 or is a further subdivision necessary? Fletcher & MacNeill (1971) distinguished a separate Strain 0 – 1 based on symptom severity to accomodate isolates that were intermediate between Strains 0 and 1. Our Group 1 includes isolates which like the Ohio strain I do not infect the *Tm*–1/*Tm*–1 differential CStMW–18 and others which like the Ohio strain II infect this host in a symptomless manner. This suggests that McRitchie & Alexander's (1973) distinction of the Ohio strains I and II should be maintained. Perhaps these Ohio strains would fall into the Strains 0 and 0 – 1 distinguished by Fletcher & MacNeill (1971).

Secondly there is the question of the systemic necrosis which may occur on Pelham's tomato hybrids with either *Tm*–2 or *Tm*–2^a. Cirulli & Alexander (1969) ascribe such necrosis to a non-specific hypersensitivity rather than to the presence of a particular strain. They established that at a high temperature regime plants heterozygous for *Tm*–2^a reacted with necrosis to four of the five strains used but not to the Ohio

Strain IV which is now called Strain 2. It is of interest to note that the more recently described Ohio Strain V was derived from Ohio Strain IV and produces a more severe necrosis than Ohio Strains I, II and III. The reaction of the isolate SJ-64 suggests that a mixture of strains may also produce a necrotic reaction. The necrosis on Craigella *Tm*-2/+ was thought to be caused by a Strain-1 fraction which was carried along with the systemic invasion by a Strain-2 isolate. In a test not previously mentioned the Strain-2^a isolate GM-65 was used either separately or in a mixture with other strains to inoculate tomato breeding lines homozygous to *Tm*-2^a. While GM-65 alone caused distinct mosaic symptoms its presence in a mixture resulted in mosaic symptoms being preceded by necrosis. These observations suggest the need for further investigation of the nature of the necrotic reactions, especially when making surveys of natural strain populations. In order to avoid difficulties with Pelham's differentials it is proposed to extend the range and include homozygous tomato breeding lines.

For the time being there is no better alternative than using the pathogenic properties of TMV strains as criteria for classification. Mosch et al. (1973) established that differences in both virulence and aggressiveness among 18 isolates were not connected with the physical and chemical properties they investigated. Neither was it possible during the present work to relate the virulence of any given isolate to its aggressiveness. Nevertheless, it is suggested that symptom characteristics should be included in descriptions of pathogenic strains. Strains with conspicuous symptoms may be used to advantage in breeding work and these certainly deserve more attention than they have received so far.

The recently introduced resistant cultivars apparently do not stand a fair chance against the variability of TMV. But it is likely that they may be grown for years to come. Since the Strains 1 and 2 occurred naturally the plant breeders have concentrated on developing cultivars homozygous for the gene *Tm*-2^a. These cultivars have been thoroughly tested during their development with a mixture of 15 isolates, five for each of the Strains 0, 1 and 2. On breeders' holdings the possibility of exposure to infections originating from susceptible tomato breeding lines was not excluded. In spite of this an adapted strain did not appear for more than three years and then MR-72 was identified as Strain 2^a. This strain was obtained from homozygous *Tm*-2^a plants which had been tested with the above mentioned strain mixture. However, some of the plants began to show symptoms after planting out so the actual source of infection could not be identified with certainty. During the growing period symptoms remained confined to the initial number of plants and did not appear in neighbouring plants of the same breeding lines. Similar observations were made in the case of GM-65 in the initial phase of its adaptation (see Table 11). This suggests that new strains may be slow in developing maximum pathogenicity. In this connection it appears unlikely that they will arise soon after resistant cultivars have been released. Exposure of these cultivars to natural infection will be insignificant compared with the test inoculum used during their development. The culture of a virus-resistant crop will tend to reduce the inoculum level. This in turn should decrease the chances of a new pathogenic strain arising.

4.8 Conclusion

Strains of TMV isolated from susceptible tomato crops in the Netherlands were similar in host range to those found in Ohio, U.S.A. The Dutch strains were identified as Strains 0, 1, 2 and 2^a (Pelham, 1968). Whereas Strain 0 and 1 may constitute the greater part of the natural population, Strain 2, and possibly, 2^a may occur as minor constituents. This subordinate position may be explained by instability of the kind shown by isolates of Strain 2 during storage in dried leaves.

In most cases isolates of the Strains 2 and 2^a were recognized only following passage through clones of *Lycopersicon peruvianum*, resistant to the strains 0 and 1 but susceptible to the strains 2 or 2^a. Theoretically it is possible that a tomato cultivar with resistance from *L. peruvianum* may sort out a strain capable of overcoming this resistance. This may depend on the time available for selection, the amount of available inoculum and on the scale on which the resistant cultivar is grown. The selection is favoured when a resistant and a susceptible cultivar are grown together and to a lesser extent when the resistant cultivar follows the susceptible in the succeeding season. On the other hand, the selection may never start if the resistant cultivar is either instantly rejected or whole-heartedly accepted. In the latter case extensive acceptance of a resistant cultivar may reduce the natural strain population to such an extent that the chances of adaptation are negligible. It is therefore possible that only resistant cultivars with outstanding cultural qualities may escape the effects of the dreadful variability of TMV.

5 Deliberate seedling inoculation with the symptomless mutant MII-16 as a means of minimizing losses caused by TMV in tomatoes

5.1 Introduction

The phenomenon of cross protection among related viruses was first demonstrated by McKinney (1929) and Thung (1931) for strains of TMV on tobacco and also applied to other viruses like potato virus X (PVX) (Salaman, 1933) and cucumber mosaic virus (Price, 1935). Since the work by Kunkel (1934) this phenomenon has been mainly used for diagnostic purposes. More recently Broadbent (1964a) suggested deliberate early inoculation of tomato crops with a common tomato strain to protect them from infection by more severe strains and to prevent fruit quality losses. His results were confirmed by Fletcher (1968) and Rast (1967c, 1968b, 1969) who reported favourably on seedling inoculation with an advance in sowing date. Jensen (1968), however, did not obtain satisfactory yields from inoculated plants compared with virus-free control plants. Attempts to cross-protect tomato crops with mild tobacco strains of TMV were only partially successful. Broadbent (1964b) established that inoculation with Holmes' masked strain (Holmes, 1934) failed to protect tomato plants against a tomato strain and did not improve yields. Rast (1969) found that plants inoculated with a mild tobacco strain isolated from his favorite brand of shag tobacco gave earlier yields but also produced some mottled fruits. Similarly, Minard et al.[1] using Holmes' masked strain found higher yields associated with a considerable number of blotchy fruits. Further progress was achieved with attenuated tomato strains produced by the prolonged heat treatment method of infected plants (Holmes, 1934). Komochi et al. (1966) obtained higher total yields from field-grown tomato plants inoculated with their strain L11. Mosaic symptoms were observed late during the growing season but only with plants which had been challenge-inoculated with a common tomato strain one week after the first protective inoculation. Paludan (1968) also reported higher total yields resulting from both early and late inoculation with an attenuated strain compared with late natural infection with a common strain.

In 1968 Rast (1972) exploiting the mutagenic action of nitrous acid on a tomato strain in crude leaf sap, isolated an almost symptomless mutant with good protective qualities. Following a brief period of experimentation the use of the mutant, MII–16, was rapidly adopted as a routine measure for seedling inoculation in commercial

1. Studies on the control of tobacco mosaic virus in glasshouse tomato crops by cross protection by H. R. G. Minard, R. A. J. White, J. Burgmans & A. D. Thomson. Unpublished reports, 1967).

tomato growing. This chapter presents a review of the various aspects connected with the practical application of this strain.

5.2 The isolation of the symptomless mutant MII-16

In order to test the possibility that mutants of TMV might be obtained from nitrous acid treatment of infective leaf sap (Sehgal, 1968) the tomato strain isolate SPS was inoculated to a batch of tomato plants in the stage of 4 – 5 true leaves. Two weeks after inoculation approximately 50 g of leaves were collected in a plastic bag and kept in a deep-freeze overnight. The tissue was then thawed and leaf sap prepared by squeezing out the juice. Following the instructions by Mundry & Gierer (1958) 2 ml of undiluted sap was placed in each of four normal glass test tubes and to each was added 1 ml 1–M sodium-acetate buffer pH 4 and 1 ml 4–M $NaNO_2$ respectively. Subsequently at intervals of 15 minutes the contents of one test tube was added to 500 ml 0.02 M phosphate buffer pH 7. The dilute preparations were used to inoculate leaves of *N. glutinosa* plants and the resulting lesions transferred singly for multiplication to one tomato seedling cv. 'Moneydor' and one young tobacco plant cv. 'Samsun'. A total number of 215 lesions was transferred in two successive experiments. Corresponding pairs of plants showing symptoms within three weeks of inoculation were discarded while those remaining symptomless were assayed on 'Xanthi nc'. Some of the latter category of plants gave negative results on assay indicating that they had escaped infection. Others were positive and developed symptoms afterwards with the exception of one pair of plants. Repeated sub-inoculations made from these symptomless plants either directly or through lesions on *N. glutinosa* to other tomato or tobacco plants consistently failed to produce symptoms. Since the original strain caused normal green mosaic symptoms it was concluded that the virus giving rise to a symptomless infection represented a mutant. The mutant was from one out of 40 lesions obtained following the 45 minutes' treatment and is referred to as MII–16.

Other mutants obtained during the experiments included those which caused either bright yellow mosaic symptoms or severe leaf distortions. It is of interest to note that two mutants showing the latter symptom were related to the tobacco strain of TMV producing a systemic reaction on the 'necrotic line' of 'White Burley' tobacco (Termohlen & Van Dorst, 1959).

5.3 The cross-protective ability of MII-16

The symptomless infections of tomato in addition to the tomato strain reaction observed on test plants made it worth considering the practical application of MII–16. First it was necessary to establish whether or not this artificial strain would effectively protect tomato plants against infection by natural strains of TMV. Preliminary tests showed that small batches of tomato seedlings inoculated with MII–16 did not produce symptoms when challenge inoculated with symptom-producing strains 10 days after the initial inoculation. Strains used for challenge inoculations included the

tobacco strain (MA), the parent tomato strain (SPS), the enation strain (ENP), the yellow ringspot strain (GdK) and the yellow mosaic strain (GPga). The results of the tests indicated that MII–16 gave sufficient protection to justify commercial scale experimentation. In this work challenge inoculation with the isolate GPga was used to assess percentage infection with mass-inoculation techniques. On several occasions it was observed that cross protection against this particular isolate became effective in less than seven days.

This did not apply to another isolate GeBl closely resembling GPga in its symptom expression. Challenge inoculation with GeBl indicated much lower rates of infection with MII–16 than did GPga. Apparently the protective action of MII–16 against GeBl required more time to develop. This observation was confirmed by the results of the tests shown in Table 15.

The test plants were at the stage of 3 – 4 leaves when first inoculated with MII–16 which was applied to the terminal leaflets of the two lowermost leaves. Three terminal leaflets of a full-grown leaf near the top of the plants were inoculated with the challenge isolates.

The results suggest that cross protection by MII–16 against yellow strains is more effective than against green strains. It should be noted that in the test of October-November 1972 the latter strains still produced symptoms when inoculated two weeks after the initial MII–16 inoculation. However, symptoms were less severe than they would have been without the protective inoculation. Similar strains defined according to Pelham's system for strain classification (1968) did not always behave identically with MII–16, e.g. GeBl as compared to GPga (Table 15).

Table 15. Symptoms resulting in plants, challenge inoculated with natural TMV strains classified according to Pelham's system (1968) after the indicated number of days following inoculation with the symptomless mutant MII–16. Three tests in 1972.

Strains used for challenge inoculation	May-June 1972				August-September				October-November			
	4	7	10	15	4	7	10	14	4	7	10	14
Green mosaic strain 0, isolate SD	+	+	+	+	–	–	–	–	+	+	+	–	+	+	+	+
Yellow mosaic strain 0, Isolate GPga	+	+	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Green mosaic strain 1, isolate SPS	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	–	+	+	–	–	+	–	+	–
Yellow mosaic strain 1, isolate GeBl	+	+	+	+	–	–	–	–	+	+	+	+	+	+	–	–	+	+	+	–	+	+	–	–
Green mosaic strain 2, isolate SL[a]	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	–	.	.	.	.	.	.	.	.
Yellow ringspot strain 2, isolate SK-68-2	+	+	+	+	+	+	–	–	–	–	–	–	–	+[1]	–	–	.	.	.	.	.	.	.	.

1. Symptoms of doubtful origin.

5.4 The growth-stunting effect of MII-16

One of the effects observed on tomato plants following early infection with MII–16 consisted of a temporary check of growth which delayed flowering and fruit set. From a practical point of view this would affect the earliness of yield. The extent of the growth-stunting effect was measured in order to get a rough estimate of the advance in sowing date necessary to compensate for this. In a series of experiments the length and fresh weight of plants were determined at weekly intervals by choosing 16 plants at random and cutting them off at the attachment of the cotyledons. Measurements were made over a 4 weeks period from approximately 5 weeks after sowing until planting-out growth stage.

The results obtained with four out of seven treatments differing in their time of infection (Fig. 15) showed that the growth rate of all treatments followed the general seasonal trend in the three successive experiments. The stunting effect of MII–16 upon growth became less pronounced with successive experiments. The advance in sowing date should therefore be varied according to the intended time of planting.

5.5 Yield experiments with MII-16

The effect of MII–16 on the yield of tomato plants was investigated in three successive years in poor growing conditions with an early winter crop. The plants for these experiments were raised in 12–cm diameter plastic pots and were transplanted into 10–l plastic pots placed on rubber dishes. Minimum night and day temperatures of 16 and 22°C respectively were maintained and the glasshouse was enriched with CO_2 during the day. Water and fertilizers were supplied by hand during the first experiment but were fed through a trickle irrigation system in later experiments. To prevent accidental infection of the control plants the experimental plots were separated by polythene screens. The regular use of diluted skimmed milk as a dip for the hands before handling the plants served the same purpose. The treatments in each experiment were replicated six times in plots consisting of either eight or four plants dependent on the number of treatments.

The first experiment early infection with MII–16 was investigated to find whether its effect would last throughout the growing period. Also if cross protection failed, how would this affect symptoms, fruit set and yield? The results of this experiment definitely indicated that early inoculation with MII–16 was superior to both an early and a late inoculation with its parent tomato strain. A challenge inoculation with the latter did not have the slightest effect on symptoms or fruit set. The experiment was prematurely ended because of the extensive occurrence of a physiological fruit disorder known as blossom-end rot (Smilde & Roorda van Eysinga, 1968). From the data collected on fruit set the earliest yields were obtained from the plants inoculated with MII–16. However, it should be noted that these plants had been sown ten days earlier than the plants intended for the late inoculation with the parent tomato strain. In this experiment the MII–16–inoculated plants were sown too early in an attempt

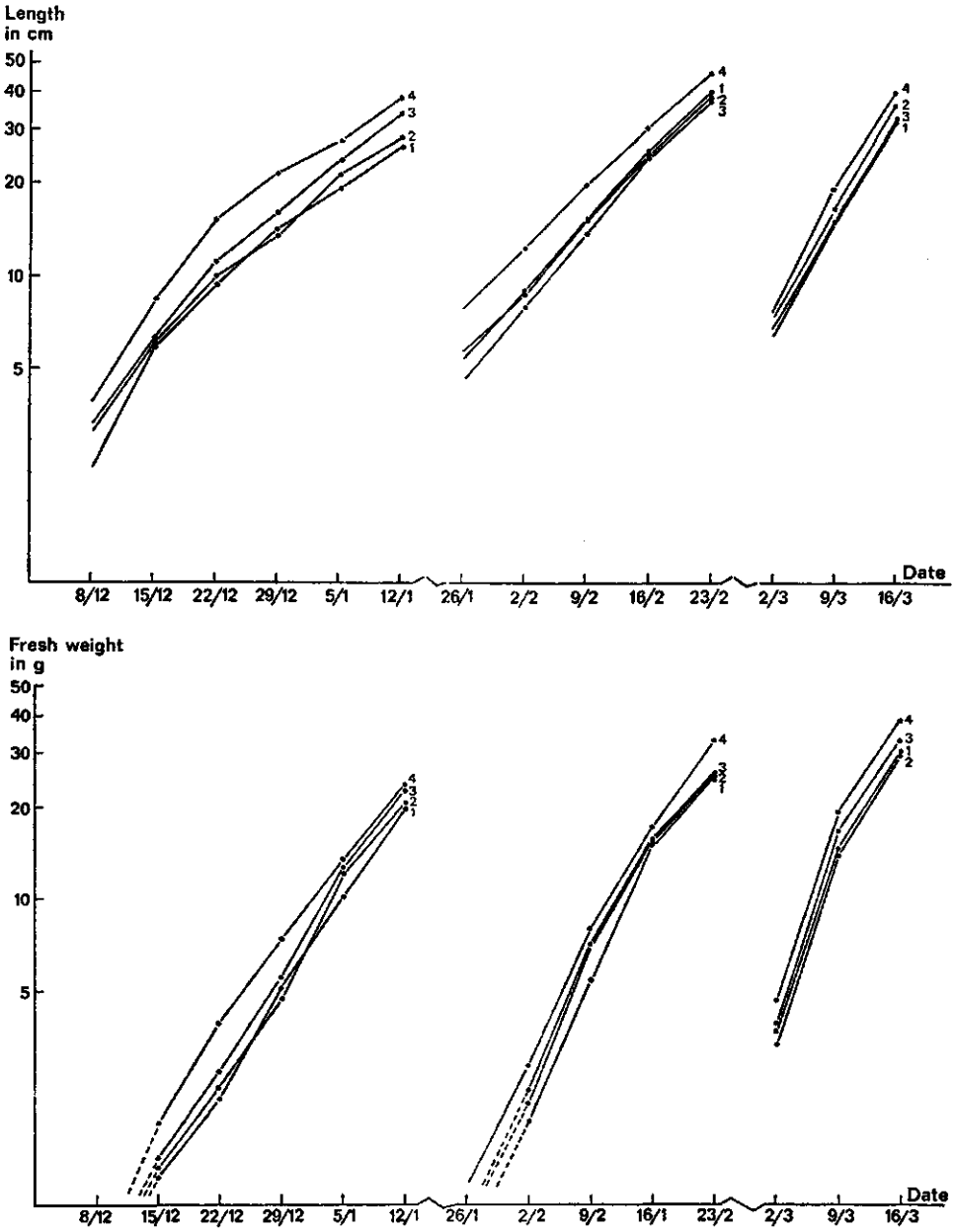

Fig. 15. The effect of an infection by the symptomless mutant MII-16 on the growth rate of plants as measured by increases in length and fresh weight. The raising method consisted of a broadcast sowing followed by transplanting seedlings into plastic pots of 12 cm diameter after 24 days. 1 = inoculated 10 days before transplanting; 2 = inoculated 1 day before transplanting; 3 = inoculated 8 days before transplanting; 4 = not oculated. First experiment: sowing date 3 November 1970, approximate planting date 6 January 1971.
Second experiment: sowing date 11 December 1970, approximate planting date 16 February 1971.
Third experiment: sowing date 19 January 1971, approximate planting date 9 March 1971.

to compensate for the stunting effect.

The second experiment was essentially similar to the first except that all the plants were sown at the same time. Again an early inoculation with MII–16 was compared with early and late inoculation with its parent strain, the latter treatment serving as a control. Late inoculation was done when fruit set on the two lowermost trusses was completed and it was found that this hardly affected yields compared with early inoculation with MII–16 (Table 16). There was a difference significant at $P < 0.1$ in the number of fruits during the last three weeks of the harvest period between early inoculation with MII–16 and late inoculation with its parent strain. Lowest yields were obtained with the parent strain and early inoculation. This treatment had significantly ($P < 0{,}05$) less weight and numbers of fruits than all other treatments throughout the harvest period. The average weight of the fruits from plants inoculated early with SPS was significantly less only during the first period of three weeks than of those obtained from other treatments. Early inoculation with SPS also significantly reduced the average number of days between setting and ripening of the fruits.

The third experiment was set up to investigate whether the early yields from inoculated plants were the result of inoculation or due to the advance in sowing date. Also

Table 16. Tomato yield and length of ripening period after early inoculation with the symptomless mutant MII–16 compared with early and late inoculation with the isolate SPS, the parent tomato strain of MII–16. Cultivar Moneydor. Sowing date of the test plants 1969/10/30.

	Early inoculation with MII–16 on 1969/12/19	Early inoculation with SPS on 1969/12/19	Late inoculation with SPS on 1970/3/4
Total number of fruits per plant			
April 25	13.2	8.1	14.0
May 16	41.3	35.4	41.0
June 6	64.2	56.6	60.2
Total yield per plant in kg			
April 25	0.77	0.45	0.81
May 16	2.44	2.09	2.48
June 6	3.74	3.31	3.57
Average fruit weight in g			
April 25	58.1	54.1	58.8
May 16	59.0	58.7	60.4
June 6	58.2	58.4	59.2
Ripening period in days	55.7	53.1	56.5

1. Average number of days between fruit set and picking.

there was evidence that in some of the previously reported tests (Rast, 1972) MII–16 had been unintentionally spread from the inoculated plants to neighbouring control plants of the same age with surprisingly favourable effects. It was also noticed that in exceptional cases the inoculated plants, without an advance in sowing date, still produced the earliest yields. The results of the third experiment were unsatisfactory in the sense that no significant differences were found between treatments in either of the two sowings. However, the late inoculation with the parent strain only produced symptoms on two out of 48 plants. This suggests that the measures taken to prevent unwanted infections were inadequate and that consequently the other plants had become infected with MII–16.

No evidence was found to support the view that in terms of yield, late inoculation should give better results than inoculation in the seedling stage. Differences in sowing date gave a significant difference in yield ($P < 0.05$) during the first three weeks of the harvest only. This confirms that the greater early yield of inoculated plants shown in previous experiments was the result of earlier sowing rather than inoculation. As there was a relatively small effect of earlier sowing date in this experiment it is questionable whether earlier sowing of plants to be inoculated is necessary. However, the method of growing tomato plants in plastic pots which confines root growth may have stimulated fruit set and so reduced differences in yield.

In order to determine the average period between flowering and maturing of fruits in each treatment a selection was made of fruits weighing between 40 and 60 g from eight specific truss positions. No significant differences were obtained but this may have been due to the almost complete failure of late inoculation with SPS. The question whether or not seedling inoculation with MII–16 induces earlier ripening of fruits remains unanswered.

The results of another experiment in commercial conditions clearly illustrated the crop insurance aspect connected with the use of MII–16. The experiment was designed to investigate the influence of seedling inoculation with either MII–16 or its parent strain on yields of tomato plants of different age. By mistake a different tomato cultivar was used for the two sowing dates so that a comparison of the effect of sowing date on early yield was not possible. The plants for this experiment were raised by a specialized plant propagator and planted out in two adjacent glasshouse-bays on two commercial holdings. The treatments were replicated four times in plots of 12 plants each. The yields were recorded by the local advisory officer, Mr B. Meyndert (Table 17 and 18). As the trials were situated in a cross-protected crop the natural infection which appeared in the control plots soon after planting out probably originated from the plants inoculated with SPS as both infected and uninfected plants were indiscriminately handled during transport. The control plants were so badly affected by TMV that they produced the lowest yields. Heaviest losses occurred with the greenback cultivar 'Extase' on holding no. 2 where yields and average fruit weights of the 'naturally' infected plants were significantly lower ($P < 0.05$) throughout the harvest period compared with those that were deliberately inoculated. The highest yields were obtained from the plants inoculated with MII–16. On holding no. 1 the differences in yield

Table 17. Tomato yield after early inoculation with the symptomless mutant MII–16 compared with early inoculation and late 'natural' infection with the isolate SPS, the parent tomato strain of MII–16. Cultivar Extase. Sowing date 1970/10/24. Inoculation date 1970/11/18.

Time of inoculation and isolate	1971/5/1		1971/6/1		Value (Dfl./m²)
	yield (kg/m²)	av. fruit weight (g)	yield (kg/m²)	av. fruit weight (g)	
Holding 1					
Early, MII–16	4.66	57.8	9.24	56.2	20.04
Early, SPS	4.06	56.4	8.88	55.4	18.38
Late, 'natural' infection SPS	3.94	57.6	8.92	57.2	18.14
Holding 2					
Early, MII–16	5.39	57.2	8.94	53.7	20.04
Early, SPS	4.68	55.1	8.41	52.2	18.27
Late, 'natural' infection SPS	2.11	50.8	5.39	46.6	10.70

Table 18. Tomato yield following early inoculation with the symptomless mutant MII–16 compared with early inoculation and late 'natural' infection with the isolate SPS, the parent tomato strain of MII–16. Cultivar Moneydor. Sowing date 1970/10/31. Inoculation date 1970/11/26.

Time of inoculation and isolate	1971/5/1		1971/6/1		Value (Dfl./m²)
	yield (kg/m²)	av. fruit weight (g)	yield (kg/m²)	av. fruit weight (g)	
Holding 1					
Early, MII–16	1.88	49.1	5.34	51.9	10.16
Early, SPS	1.46	49.3	4.80	52.3	8.87
Late, 'natural' infection SPS	1.41	47.4	4.86	51.2	8.92
Holding 2					
Early, MII–16	2.42	49.8	5.47	47.2	11.01
Early, SPS	1.99	48.9	5.20	48.0	10.39
Late, 'natural' infection SPS	1.80	49.1	4.73	45.1	9.23

were significant throughout the harvest period whereas on holding no. 2 they were significant only during the first part. The best financial returns are linked with earliness and the total fruit yield. In this respect seedling inoculation with MII–16 was most successful. With the greenback-free cultivar 'Moneydor' on holding no. 2 any inoculation treatment proved significantly more profitable than chance infection of the control treatment.

The experimental results obtained so far indicate that MII–16 is certainly preferable to a common strain for seedling inoculation. Also that there is no distinct advantage is using MII–16 for late inoculation compared with seedling inoculation. Considering that natural infection of tomato crops with common strains may occur prematurely it is better to avoid unnecessary losses in earliness and total yield by seedling inoculation with MII–16.

Table 19. The effect of dry-heat treatment on the internal virus contents of seeds[1] derived from plants inoculated in the seedling stage with either the symptomless mutant MII-16 or the parent tomato strain, the isolate SPS.

Treatment	Source plant inoculated with:	
	MII-16	SPS
Seeds not heat-treated	18	294
Seeds treated at 76°C for 1 day	0	194
Seeds treated at 76°C for 2 days	1	94
Seeds treated at 76°C for 3 days	3	69

1. Expressed as total number of lesions appearing on assay on 72 half leaves of *Nicotiana glutinosa* i.e. 12 sub samples each on 6 half leaves.

5.6 Internal seed infection caused by MII-16

The favourable effects of seedling inoculation with MII-16 on yields resulted in its use by a number of seed growers in order to increase seed production. Furthermore it was thought that such practice might eliminate common strains of TMV from the seed, making heat treatment unnecessary. To test this latter possibility fruits from the lowermost trusses of plants which had been inoculated in the seedling stage with either MII-16 or its parent tomato strain were harvested for their seeds. Following a normal process of fermentation the freshly cleaned and dried seeds were treated with a Na_3PO_4 solution to disinfect them externally, rinsed thoroughly in running tap water and dried. Subsequently, samples of 100 seeds were subdivided by cutting single seeds into four to form four smaller samples presumed to have equal virus contents. Four smaller samples of 25 whole seeds would not have ensured equal virus content per sample because each of the samples might have contained a different number of internally infected seeds (Broadbent, 1965b). Three corresponding samples of fragmented seeds were put separately into 2-ml glass tubes and heat treated for either 1, 2 or 3 days, the remaining sample was left untreated. For each of the virus strains involved 12 samples of 100 seeds were treated this way. Following heat treatment the fragmented seed samples were ground in a mortar with a small amount of fine, sterilized sand and 3 ml of water. This preparation was then used to inoculate six random half leaves of *N. glutinosa*.

The results of these assays are presented in Table 19 and confirm that seedling inoculation with MII-16 results in a negligible internal infection of seeds and may therefore appear as an alternative to heat treatment. It should be noted that the results in general confirm those obtained by Steepy (1968) who found a lower incidence of seed infection in connection with a heat-attenuated strain (Paludan, 1968) compared with the normal tomato strain.

5.7 Undesirable properties of MII-16

Some time after its isolation and first successful performance in commerce MII–16 was found to possess some properties which could make its commercial performance unreliable. The original isolate of MII–16 was stored in samples of dried tomato and tobacco leaves. After one and a half years of storage it was discovered that when a dried leaf fragment was used to inoculate *N. glutinosa* and single lesions were transferred to 'Samsun' tobacco only 7 out of 40 plants remained symptomless whereas the others developed mosaic symptoms. Apparently the symptomless mutant in the mixture had been inactivated to a much greater extent than the symptom-producing strains. Although the proportion of the latter was reduced considerably by repeated single-lesion isolation it was never completely eliminated. The poor keeping quality of MII–16 suggested the use of concentrated purified suspensions which would enable large amounts of inoculum to be stored in frozen condition. The methods applied in preparing such inocula and in testing them prior to their release for commercial use have been described in the appendix to this work.

A further problem with MII–16 occurred in the summer of 1973 when it was almost impossible to extract virus from inoculated tomato plants. This applied to plants inoculated in the seedling stage with bulk inoculum or individually with single lesions from *N. glutinosa*. In the latter case simultaneously inoculated 'Samsun' plants yielded measurable quantities of virus. Whether the low rate of multiplication of MII–16 was due to a temperature sensitivity as observed with other nitrous acid mutants (Jockusch, 1964) or other factors awaits further investigation.

5.8 Mass inoculation techniques

In the period when MII–16 was tried on numerous private holdings manual inoculation of tomato seedlings sometimes resulted in spread of seed-transmitted natural strains of TMV. Apart from this risk manual inoculation of many thousands of seedlings was a time consuming and tiresome job. Therefore inoculation with a spray gun was tried (Marrou & Migliori, 1965) in order to find a method, which at reasonable costs, would guarantee as near to 100% infection of the seedlings as possible. Several technicalities such as the dilution of the inoculum, the amount of abrasive to be added, the type of equipment, etc. had to be resolved before manual inoculation could be superseded.

For the experiments batches of 25 seedlings raised from virus-free seeds were pricked out in normal potting soil contained in styropor boxes measuring 45 × 35 × 6 cm each. There were four replicates per treatment and infection with MII–16 was assessed following a challenge inoculation with the yellow mosaic strain. The basic inoculum of MII–16 was a purified virus suspension which was used in a dilution of 1:1000 or 1:10,000 for these experiments. Carborundum was generally used as an abrasive, celite being tested only twice. A Sprio SM–63 spray gun was used for the inoculation as this gun allowed for an adjustment of the angle of the fan jet. The press-

ure was supplied by a tank of compressed air. This equipment was compared to a Binks electric compressor-spray gun unit (gun no. 35) and a Wagner Mistral type 300 airless sprayer with a built-in pump. The latter type was put out of order by the abrasive in the inoculum after two experiments. The spray-inoculations were performed by the professional painter employed at the station. The results of the various inoculation treatments have been compiled in the Tables 20, 21 and 22. The same stock of inoculum of MII–16 was used in the three experiments reported in Tables 20 and 21.

Older seedlings may be inoculated in a cheap, yet efficient, way simply by wetting them with inoculum before pricked out (Table 20). Such a method, however, is not likely to be accepted by growers who would prefer to prick out dry seedlings. Brushing the wetted seedlings by hand saves time but it appears to be far less effective than fingering them one by one. A battery-operated truss vibrator used to induce infections with the older seedlings did not give satisfactory results probably because this tool repeatedly failed working.

The results in Table 21 show that spray inoculation may be as effective as manual inoculation. Spray inoculation, however, requires a much greater amount of inoculum most of which is wasted during the operation. It was found that 5 ml of inoculum when diluted 1:1000 was sufficient for thorough treatment of about 10,000 seedlings

Table 20. Percentage infection on different methods of manual inoculation of tomato seedlings with the symptomless mutant MII–16 as assessed by challenge inoculation with the isolate GPga of the yellow mosaic strain. The inoculum of MII–16 diluted 1:1000 and containing 20 g carborundum 500 mesh per 1 was sprayed onto the seedlings.

	Percentage infection		
	Exp. 1	Exp. 2	Exp. 3
1. Inoculation of seedlings 10 days after germination by pricking out when still wet	.	28	87
2. Inoculation of seedlings 10 days after germination by pricking out when dried up	6	.	28
3. Inoculation of seedlings 20 days after germination by pricking out when still wet	.	90	100
4. Inoculation of seedlings 20 days after germination by pricking out when dried up	57	69	100
5. Inoculation of transplanted wetted seedlings 15 days after germination by brushing 3 × with the hand palm	32	77	75
6. Inoculation of transplanted wetted seedlings 15 days after germination by rubbing cotyledons between thumb and index finger	96	100	100
7. Inoculation of transplanted wetted seedlings 15 days after germination by touching them with truss vibrator[1] in operation	99	.	.
8. Inoculation of transplanted wetted seedlings 25 days after germination by touching them with truss vibrator[1] in operation	76	.	.

1. Normally used for pollination of flowers to improve fruit set.

Table 21. Percentage infection on different methods of spray inoculation of tomato seedlings with the symptomless mutant MII–16 as assessed by challenge inoculation with the isolate GPga of the yellow mosaic strain.

Treatment	Percentage infection		
	Exp. 1	Exp. 2	Exp. 3
Spray gun Sprio SM–63 in combination with compressed air adjusted to produce 1 ato at entrance into gun. Working distance 20 – 30 cm. plants hit twice			
1. Inoculum diluted 1:1000, 20 g carborundum per l	98	100	100
2. Inoculum diluted 1:1000, 5 g carborundum per l	98	91	83
3. Inoculum diluted 1:10000, 20 g carborundum per l	96	86	61
4. Inoculum diluted 1:10000, 5 g carborundum per l	81	92	7
5. As Treatment 3 but repeated after 3 days	99	100	26
6. As Treatment 4 but repeated after 3 days	94	88	17
7. Inoculum diluted 1:1000, carborundum dusted on seedlings previous to spraying	100	99	80
Airless sprayer Wagner Mistral type 300. Working distance 10 cm. Plants hit twice			
8. Inoculum diluted 1:1000; carborundum dusted on seedlings previous to spraying	.	99	69
9. Inoculum diluted 1:1000; 20 g carborundum per l	.	98	91

spaced 5 cm apart after being pricked out into small soil pots. Attempts to use the inoculum more economically in Treatments 3 – 6 gave promising results in Experiments 1 and 2 but failed to do so in Experiment 3. For the irregularities observed there is no plausible explanation as the spraying was done meticulously. Considering that the same stock of deep-frozen inoculum was used it is hard to believe that its infectivity deteriorated as a result of the instability of MII–16 within the period of four months covered by the experiments. In order to ensure as high a percentage infection as possible it is better to recommend higher concentrations of MII–16 and abrasive than is strictly necessary. Dusting the seedlings with carborundum previous to spraying them was done to find a successful method of application for the inexpensive Wagner Mistral without damaging it. It was realized, however, that such a method involves a risk to the health of the operator as a result of inhaling the carborundum dust.

Carborundum sometimes caused clogging of the spray gun but in an experiment to investigate whether celite would be more suitable it was found that both are equally effective provided they are kept suspended in the inoculum by shaking the spray gun occasionally during inoculation (Table 22). Although differences between Treatments 1 – 12 are small one may conclude that celite would be preferable as it settles at a slower rate than carborundum and can be used in smaller amounts. The results also suggest that it is worth hitting the plants hard with the fan jet of the spray gun adjusted to the narrowest possible angle. In this connection the Sprio is better than

Table 22. Percentage infection on different methods of spray inoculation of tomato seedlings with the symptomless mutant MII–16 as assessed by challenge inoculation with the isolate GPga of the yellow mosaic strain.

Treatment	Percentage infection
Spray gun Sprio SM–63 in combination with compressed air adjusted to produce 2 ato at entrance into gun. Working distance 15 – 20 cm. Inoculum diluted 1:1000	
1. Carborundum 20 g[1] per 1; fan jet 55 – 60° ; plants hit twice	100
2. Carborundum 20 g per 1 ; fan jet 55 – 60° ; plants hit once	95
3. Carborundum 20 g per 1 ; fan jet at its widest; plants hit twice	89
4. Celite 4 g[1] per 1 ; fan jet 55 – 60° ; plants hit twice	96
5. Celite 4 g per 1 ; fan jet 55 – 60° ; plants hit once	99
5. Celite 4 g per 1 ; fan jet at its widest; plants hit twice	95
7. Celite 2 g per 1 ; fan jet 55 – 60° ; plants hit twice	99
8. Celite 2 g per 1 ; fan jet 55 – 60° ; plants hit once	88
9. Celite 2 g per 1 ; fan jet at its widest; plants hit twice	94
10. Celite 1 g per 1 ; fan jet 55 – 60° ; plants hit twice	96
11. Celite 1 g per 1 ; fan jet 55 – 60° ; plants hit once	91
12. Celite 1 g per 1 ; fan jet at its widest; plants hit twice	90
Binks spray gun no. 35 compressor unit said to produce a maximum pressure of 3 ato. Working distance 20 cm. Fan jet adjusted to include narrowest possible angle. Plants hit twice	
13. Inoculum diluted 1:1000; carborundum 20 g per 1	74
14. Inoculum diluted 1:1000; celite 4 g per 1	69

1. These quantities take about the same volume.

the Binks outfit which hardly allows for such regulation. The steady pressure resulting from the use of compressed air with the Sprio is not necessarily better than the jerky pressure produced by the Binks compressor.

The results of these experiments and the experience gained from many other commercial applications served as a basis for the recommendations issued for commercial application of MII–16. These included the use of a spray gun with a working pressure of 3 atmosphere to be held at a distance of 15 to 20 cm from the tomato seedlings during inoculation. The purified virus suspensions distributed in quantities of 5 ml were to be used as inocula in a dilution of 1:1000 with 100 g of carborundum 500 mesh added as an abrasive.

5.9 An evaluation of the use of MII-16 in commercial crops of tomato

The introduction of MII–16 into commercial practice went smoothly. Although in general MII–16 performed successfully it did not always come up to expectations. Some of the failures experienced were due to contamination of the inoculum by symptom-producing strains including the parent tomato strain. In this connection the incidence of normal mosaic symptoms was observed to be three to seven times

higher with inoculation before pricking out than with an inoculation afterwards. In the former case the seedlings contracting the mosaic strain apparently acted as a source of infection for other seedlings for a few days before the symptomless mutant had time to exert its protective action. The growers were therefore advised to delay inoculations until the seedlings had resumed growth after being pricked out into pots. This measure had the additional advantage that the plants would suffer least from the renewed stunting of growth caused by MII-16.

Strains of TMV originating from infected seeds or contaminated seed coats sometimes presented difficulties. Serious cases of seed transmission were often traced to popular recently introduced cultivars where only freshly harvested seeds were available. Some of the smaller seed producers still do not practice any kind of disinfection, so private growers were encouraged to treat their seeds with Na_3PO_4 to avoid unnecessary risks. Plants inoculated in the seedling stage with MII-16 produced seeds that were practically free from internal virus (see section 5.6). But seed growers could not be safely advised to inoculate while the effects of the contaminating strains in the inoculum and any sensitivity to high temperatures were unknown.

The source of the TMV causing mosaic symptoms in inoculated crops may be difficult to establish. If symptoms appear it is first attempted to find out whether the crop became protected by inoculation with MII-16. This is done by examining apparently healthy plants for scattered yellow spots on the older leaves. Such spots may contain yellow strains possibly arising from MII-16 by mutation. If they are missing it may be concluded that the inoculation was not effective. It is possible that the inoculum may have contained too low a concentration of MII-16. When preparing the purified suspensions from infected plant material variations in virus content could not be taken into account. On the other hand inoculum was not always correctly applied or was used for twice as many plants as recommended. For whatever reason, such mosaic symptoms may have originated in a number of ways. If faulty inoculation is ruled out then contamination of the inoculum or seed transmission are possible reasons for symptom appearance. In either case competition between the symptomless mutant and the mosaic producing strains occurs with the symptom producing isolate becoming dominant because of the slow rate of multiplication of MII-16. This would account for a gradual development of mosaic symptoms in scattered plants throughout the crop with fruit set hardly affected. One can only speculate on the influence of external factors on the interaction between strains. The tendency of mosaic symptoms to occur more frequently in autumn crops than in earlier planted crops may be explained by a detrimental effect of high temperatures on the multiplication of MII-16.

The discussion of the difficulties which may arise from seedling inoculation with MII-16 was not intended to discredit the technique. On the contrary it should be stressed that the difficulties were an exception rather than the rule; MII-16 inoculation of tomatoes was very well received by the growers and is still being used by a large majority. Records published by the Agriculture Economics Research Institute (L.E.I.) indicate that for early heated crops the average yields per m^2 increased from 9.79 kg in 1971 to 10.78 kg in 1972 and to 11.28 kg in 1973 (L.E.I.-informatie, 1973).

Improvements in methods of CO_2 enrichment and trickle irrigation may also have contributed to this development. However, the cultural techniques do not explain the striking increase of yields in 1972, the year in which MII–16 was officially released for commercial use.

It is regrettable that seedling inoculation with MII–16, which was mainly intended as a transitional method of control, may now be an obstacle to the introduction of resistant cultivars. Growers are reluctant to accept resistant cultivars when seedling inoculation enables them to take the fullest possible advantage of their old trusted cultivars. New TMV-resistant cultivars have not yet outyielded inoculated susceptibles and the unsatisfactory cultural qualities of former resistant selections has also discouraged growers. It is of interest to note that a number of TMV-resistant cultivars have been readily accepted not because of their resistance to TMV but because of resistance to *Fusarium*. It is a comforting thought that, whenever necessary, the same variability of TMV which may give rise to pathogenic strains capable of overcoming present-day resistance may also provide suitable strains to protect them by seedling inoculation.

5.10 Concluding remarks

The information given on the symptomless mutant, MII–16, may appear somewhat fragmentary. It should be realized, however, that once its protective qualities were known an acceptable solution to the ever present problem of tomato mosaic was brought within easy reach. Therefore, investigations were concentrated on matters which were considered essential for the successful application of MII–16 in commerce. The growth-stunting effect was assessed in order to adjust the data of sowing and of inoculating seedlings in relation to methods of plant raising and season. Demonstrations arranged on 54 private holdings throughout the growing area gave satisfactory results which stimulated the growers' interest (Rast, 1972). This made it necessary to undertake large scale production of inoculum and to develop mass inoculation techniques.

The occurrence of contaminating symptom-producing strains in a dried leaf sample of the original isolate added an unexpected complication. As a consequence inocula had to be supplied as purified suspensions which were first tested for contamination and infectivity. Moreover, a sufficient quantity of inoculum had to be prepared and stored in case the whole of the tomato crop, which exceeds 3000 ha, should be treated. All this limited the time available for a complete virological characterization of the strain MII–16. The possible role of MII–16 in preventing internal seed infection by the normally occurring strains of TMV or its possible temperature sensitivity have yet to be investigated.

In general it may be concluded that the practical results obtained with the symptomless mutant, MII–16, have been very satisfactory. Apart from ensuring increased yields cross protection has saved growers labour spent attempting to check the spread of tomato mosaic.

6 General discussion

From the preceding pages it is evident that various strains of TMV may occur in susceptible crops of tomato. As far as symptoms are concerned a rough distinction can be made between green mosaic, yellow mosaic and necrosis strains. When differentiated on suitable test plants the green mosaic strains are divisable into tomato and tobacco strains with the former predominant. The properties of these two strains in vitro do not explain the dominance of the tomato strain as they are both very similar with regard to infectivity and stability. They would appear to have equal chances of persistence in tomato seed and root debris and therefore to start new infections in tomato crops. The tomato plant may be more susceptible to the tomato strain enabling it to multiply and move in the plant at a faster rate than the tobacco strain (Komuro et al., 1966). It has been demonstrated that in tomato plants the tobacco strain even when inoculated before the tomato strain is eventually superseded by the latter. For this reason it is doubtful whether the tobacco strain from smoking tobacco is of significance in tomato crops especially as it is a poor source of inoculum (Broadbent, 1962; Komuro & Iwaki, 1968). The results obtained with nitrous acid treatment of tomato strain isolates have shown that other mutants occur and some of these caused a reaction typical of the tobacco strain. It is possible that in nature the tomato strain may produce the tobacco strain by mutation but, if it does, such mutants are recovered very infrequently.

The infrequent occurrence of strains in practice may be explained by their instability as established during storage in dried leaves. A marked example is the isolate SL^b obtained from light-coloured lesions caused on *Petunia hybrida* by the winter necrosis strain SL^a. The isolate SL^b had such a low infectivity that it was extremely difficult to maintain a culture which consistently produces characteristic local necrotic lesions on 'Samsun' tobacco. The reproduction of these lesions was only possible during the winter following inoculations from necrosis on leaves or stems of 'Samsun' or from lesions on *Nicotiana glutinosa*. Necrotic local lesions were not produced with inocula made from systemically infected leaves showing different symptoms. The original sample of TMV designated as SL was obtained from a tomato plant affected by necrosis known as 'streak'. The large number of isolates derived from this original sample included the series SL^a, SL^b and SL^c of which the latter gave symptoms typical of the yellow mosaic strain. This suggests that SL^b is either just a mixture of strains or a highly unstable strain which by mutation may produce various strains (Norval, 1938).

The strains differentiated on an experimental host range and afterwards identified as Strains 0, 1 and 2 (Pelham, 1968) were also different in their stability in vitro. When stored in dried leaves the infectivity of Strains 0 and 1 was fully preserved whereas that of Strain 2 was partially lost. The loss of infectivity was not common to all hosts but to *Lycopersicon peruvianum* which being resistant to Strains 0 and 1 should have been susceptible to Strain 2. Fortunately, the capacity of Strain 2 to cause visible infections on *L. peruvianum* was retained by purified suspensions kept in frozen condition. This again suggests that a mixture of strains was involved rather than a pure strain. It is not known what would have happened to the infectivity of Strain 2 if infected leaves of *L. peruvianum* had been used for dry storage instead of leaves of 'Samsun' tobacco.

When finally assessing the possibility of controlling tomato mosaic the choice is definitely in favour of growing resistant cultivars compared with cross protection. The strains capable of overcoming the resistance derived from *L. peruvianum* represent a minor constituent of the natural strain population occurring in susceptible crops. Presumably being relatively unstable such strains may not survive long enough to become a real threat to resistant crops. On the other hand, the success of cross protection depends on a symptomless mutant, MII–16, possessing the instability of the isolates of Strain 2 referred to above. Furthermore, the symptomless mutant occurs in a mixture with symptom producing strains which may have come into existence as a result of further mutations as suggested with the isolate SL^b. Continuous efforts will be necessary to get rid of such contaminating strains in the inocula of MII–16. These efforts are rewarding as shown by the satisfactory results obtained so far with this method of control. If, as a result of the variability of TMV the present-day resistance is overcome, cross protection might be the only alternative left. Tomato growers will not be content with levels of yield below those achieved by either cross protection or by growing resistant cultivars.

Summary

Tomato mosaic, which is caused by tobacco mosaic virus (TMV) has always been a problem of tomatoes grown under glass. This is due to the infectivity and also the persistence of the virus. Control may be achieved by preventing infection either by taking preventive measures or by growing resistant tomato varieties. There is also the possibility of minimizing the damage caused by tomato mosaic by deliberate seedling inoculation. Many strains of the virus occur. In this work the significance of this variability is considered in relation to the different approaches for controlling tomato mosaic.

From a study of the literature concerning the persistence of the virus in tomato seeds and in the soil it appears unlikely that infection of susceptible tomato crops can be prevented. With tomato seeds the virus may occur both internally and externally and is distributed very irregularly among different batches of seeds. A tomato seedling may become infected from the seed only when being handled during pricking off. Heat treatment of seeds for 24 hours at 80°C may not have harmful effects in germination but it is sometimes inadequate to inactivate the virus completely. Tomato plants may also become infected by contact with debris of a previous crop which has remained on or in the soil. The temperature required for the inactivation of the virus is not reached in every part of the soil with the current methods of soil sterilization. With the sheet method of steaming temperatures at depths below 30 cm often remain lower than 80°C. This is not sufficient to inactivate the virus. Recent developments in commercial practice such as sowing pelleted tomato seeds separately in small soil pots and steaming the soil through a permanent system of drain pipes, make it feasible to grow tomatoes free from TMV. However, increased yields may not compensate for the higher labour costs of further measures to prevent infection.

Symptoms are described of eight different TMV strains which may occur in susceptible tomato crops. Of the strains which cause normal mosaic symptoms the tomato strain is by far the most important and the tobacco strain is uncommon. These two strains may be differentiated on a special selection of *Nicotiana tabacum* cv. White Burley. When tomato plants are inoculated either simultaneously or successively with both strains usually the tomato strain is isolated afterwards. The tobacco strain when first introduced is replaced wholly or partially by the tomato strain. The tobacco strain in tomato crops may have originated from smoking tobacco and it is assumed that the other strains arise as mutants from the tomato strains. From the reaction of test plants they seem more closely related to the tomato strain than to the tobacco strain. As well as the strongly distorting enation strain, the yellow ringspot

strain and the yellow mosaic strain necrosis-inducing strains also occur in tomato. Unstable representatives of the latter are possibly involved with the phenomenon of 'single virus streak'. This symptom, which is characterized by necrosis particularly affecting early heated crops of tomato in the spring, has not been reproduced experimentally. A new strain was found ,which on account of characteristic fruit symptoms, has been named the crusty fruit strain. The strains which are clearly distinct from the tomato strain are a small minority. In attempts to prevent infection of a susceptible tomato crop they are of no significance.

Breeding for resistance is reviewed in relation to strains. Strains with striking symptoms may be useful for testing purposes. However, in breeding for resistance it is important to have strains of TMV which vary in their capacity to infect resistant plants. In order to demonstrate such differences clonal test plants were used of *Solanum pennellii, Lycopersicon esculentum* breeding line CStMW–18 and a number of *L. peruvianum* accessions. On this differential host range four strains 0, 1, 2 and 2^a were distinguished which fit into Pelham's system for strain classification (1968). Strain 0 causes only symptoms on susceptible plants, Strain 1 also on those with the gene for tolerance *Tm*–1 (*L. esculentum* CStMW–18) or comparable genes (*S. pennellii*). Strains 2 and 2^a cause symptoms not only on susceptible plants but also on plants with the genes *Tm*–2 and *Tm*–2^a respectively. Tests with isolates of strains 2 and 2^a on *L. peruvianum* initially gave inconsistent results. Passage through susceptible *L. peruvianum* plants caused an increase in the infective capacity of the isolate involved until it consistently infected a certain number of *L. peruvianum* clones. The stabilizing influence of the host on the infectivity of the virus, which may be defined as 'selection pressure' (Robinson, 1969) sometimes requires a considerable period of time to become apparent. In the case of the isolate GM–65 one of the originally infected plants was kept alive for two years before an isolate of Strain 2^a was found in it. Most isolates of Strain 2 were unable to infect *L. peruvianum* after storage in dried leaves. By contrast this infective capacity was preserved by storage in deep-frozen suspensions. Strain 1 isolates did not lose their characteristic infectivity towards *L. esculentum* CStMW and *S. pennellii*. Whereas Strain 0 is very common in susceptible tomato crops, Strains 1 and 2 occur much less frequently. Strain 2^a is considered extremely rare. Although the rapid increase in use of resistant tomato cultivars, homozygous for *Tm*–2^a, may allow Strain 2^a to diverge from the natural collection of strains, such evolution is not likely to occur soon.

Finally the practical application of inoculation with the symptomless nitrous acid mutant, MII–16, is discussed. Although infection of tomato plants with MII–16 causes some stunting of growth it protects them adequately against later infections with other strains of TMV. In yield experiments it has been established that treatment with MII–16 ensures optimal yields from susceptible tomato varieties. There are also indications that the mutant could be used to obtain virus-free seeds. Further it has been established that MII–16 is actually a mixture of strains consisting of the symptomless strain and another strain resembling the parent tomato strain. Compared with the latter the mutant multiplies at a slower rate and is more readily inactiv-

ated by storage in dried leaves. For these reasons it is necessary to repeatedly reisolate the mutant from the strain mixture and to supply inocula for commercial use as purified suspensions which may be kept in frozen condition.

Infection by MII–16 may be verified by a challenge inoculation with a strain of TMV causing yellow mosaic symptoms on plants not infected by the mutant. This method may be used both in developing mass inoculation techniques and in testing inocula for infectivity prior to commercial use. Normal mosaic symptoms which may occur in commercial practice in spite of treatment with MII–16 may result from contamination of the inoculum or infection by other strains from seeds or incomplete infection with MII–16 because of inaccuracy during inoculations. In general cross protection with MII–16 has given satisfactory results. This experience proves that the variability of TMV which threatens resistant tomato cultivars may be satisfactorily exploited for cross protection.

Acknowledgments

The author wishes to express his sincere gratitude to Prof. Dr J. P. H. van der Want for the interest taken in the investigations and for the guidance given during the preparation of the manuscript.

He is greatly obliged to Boards and Directors of the Institute for Phytopathological Research, Wageningen, and the Glasshouse Crops Research and Experiment Station, Naaldwijk, for allowing him a free hand in conducting his research and for granting him the time to work on this manuscript.

The author is much indebted to Dr J. T. Fletcher of the Agricultural Development and Advisory Service, Leeds, for his valuable suggestions and for correcting the English text.

Many thanks are also due to Miss Drs F. Quak and to Dr L. Bravenboer for critically reading through the draft of the manuscript and their valuable comments.

Samenvatting

Het tomatemozaïek dat veroorzaakt wordt door het tabaksmozaïekvirus (TMV) is steeds een probleem geweest voor de tomatenteelt onder glas. Dit is te wijten aan de karakteristieke eigenschappen van het virus, dat zeer besmettelijk en tevens zeer bestendig is. De bestrijding kan gericht zijn op het voorkomen van infectie, hetzij door het treffen van sanitaire maatregelen of door het telen van resistente tomaterassen. Verder bestaat de mogelijkheid om door een opzettelijke kiemplantinfectie aan de meest schadelijke gevolgen van het tomatemozaïek te ontkomen. Van het virus komen vele stammen voor. In dit proefschrift wordt nagegaan welke betekenis deze variabiliteit van het TMV heeft voor de verschillende wijzen waarop de bestrijding van het tomatemozaïek benaderd kan worden.

Eerst worden aan de hand van een literatuurstudie betreffende het overblijven van het virus in tomatezaad en in de grond aangetoond dat het nauwelijks mogelijk is om infectie van een vatbaar tomatengewas te voorkomen. Het virus kan bij tomatezaad zowel in- als uitwendig voorkomen en is zeer onregelmatig verdeeld over verschillende partijen zaad. De overbrenging van het virus uit tomatezaad vindt alleen plaats bij het verspenen van kiemplanten, bijvoorbeeld door contacten met geïnfecteerde zaadhuidjes. Een warmtebehandeling van zaad tot 80°C gedurende 24 uur wordt zonder schade voor de kiemkracht verdragen, maar is soms niet afdoende om al het virus te inactiveren.

Tomateplanten kunnen ook geïnfecteerd raken door contacten met resten van een vorig gewas welke op of in de grond zijn achtergebleven. Voor de inactivering van het virus worden de vereiste temperaturen bij de gangbare methoden van grondstomen niet overal in de grond bereikt. Bij het zogenaamde zeilstomen blijven de temperaturen op grotere diepten dan 30 cm vaak lager dan 80°C. Dit is niet voldoende om naderhand wortelinfecties te voorkomen.

Recente ontwikkelingen in de praktijk zoals het afzonderlijk in kleine grondpotten uitzaaien van ingehulde tomatezaden en het grondstomen door een permanent stelsel van drainagebuizen maken het virus-vrij telen van tomaten uitvoerbaar. De vooruitzichten zijn echter niet aantrekkelijk omdat de meerdere opbrengsten waarschijnlijk niet zullen opwegen tegen de hogere arbeidskosten verbonden aan verdere maatregelen ter voorkoming van infectie.

Vervolgens worden de symptomen beschreven van acht verschillende TMV-stammen welke in vatbare tomatengewassen kunnen voorkomen. Van de stammen welke normale mozaïeksymptomen veroorzaken, neemt de tomatestam in vergelijking met de tabaksstam verreweg de belangrijkste plaats in. De genoemde stammen laten zich

op onder andere een bijzondere selectie van *Nicotiana tabacum* cv. 'White Burley' van elkaar onderscheiden. Uit tomateplanten, welke gelijktijdig of opeenvolgend met beide stammen zijn geïnoculeerd wordt later in hoofdzaak de tomatestam geïsoleerd. Waar de tabaksstam als eerste is ingebracht wordt deze geheel of grotendeels verdrongen door de tomatestam. Terwijl de tabaksstam in tomategewassen bijvoorbeeld uit tabak afkomstig zou kunnen zijn, wordt van de overige stammen aangenomen dat ze als mutant uit de tomatestam ontstaan. Uit de reactie van toetsplanten lijken ze nauwer verwant te zijn aan de tomatestam dan aan de tabaksstam. Behalve de sterk misvormende enatiestam, de gele kringvlekkenstam en de geelmozaïekstam komen op tomaat ook necrotiserende stammen voor.

Het is mogelijk dat minder stabiele vertegenwoordigers van de laatstgenoemde categorie betrokken zijn bij het niet geheel reproduceerbare verschijnsel van de zogenaamde 'strepenziekte'. Deze is gekenmerkt door stengelnecrose welke in het voorjaar incidenteel voorkomt in vroege stookteelten van tomaat. Verder is een stam gevonden welke naar de karakteristieke vruchtsymptomen de vruchtkorstenstam is genoemd.

Als geheel vormen de stammen welke duidelijk van de tomatestam zijn te onderscheiden slechts een kleine minderheid. Bij een preventieve bestrijdingswijze in een vatbaar tomategewas hebben ze geen enkele betekenis.

Verder wordt een overzicht gegeven van het stammenonderzoek ten behoeve van toetsingen bij de resistentieveredeling. Van stammen met opvallende symptomen kan voor dat doel een nuttig gebruik worden gemaakt. Voor de veredeling op resistentie is echter van wezenlijk belang dat TMV-stammen ook kunnen verschillen in het vermogen om resistente planten te infecteren. Om dergelijke verschillen aan te tonen zijn stekplanten gebruikt van *Solanum pennellii*, de *Lycopersicon esculentum* selectie CStMW–18 en een aantal *L. peruvianum*-herkomsten. Op deze differentiële waardplantenreeks zijn vier stammen te onderscheiden welke als de stammen 0, 1, 2 en 2^a passen in het classificatiesysteem van Pelham (1968). Stam 0 veroorzaakt alleen symptomen op vatbare planten, stam 1 ook op die met het tolerantiegen Tm^{-1} (*L. esculentum* CStMW–18) of daarmee vergelijkbare genen (*S. pennellii*). De stammen 2 en 2^a veroorzaken behalve op vatbare planten alleen symptomen op planten met de respectievelijke resistentiegenen *Tm*–2 en *Tm*–2^a (*L. peruvianum*).

Met isolaten van stam 2 en 2^a gaf de toetsing op *L. peruvianum* aanvankelijk sterk wisselende resultaten. Passage door vatbare *L. peruvianum*-planten deed het infectievermogen van het betrokken virus toenemen totdat het constant een zekere waardplantenreeks van genoemde soort kon infecteren. Een dergelijke stabiliserende invloed van de waardplant, welke als 'selectiedruk' omschreven kan worden, moet soms geruime tijd op het virus inwerken. In het geval van het isolaat GM–65 moest één van de eerst geïnfecteerde planten twee jaar worden aangehouden, voordat het virus op andere planten kon worden overgebracht. Toen was het pas mogelijk om GM–65 met stam 2^a te identificeren.

Een aantal isolaten van stam 2 bleek het infectievermogen ten aanzien van *L. peruvianum* bij bewaring in gedroogd blad te verliezen. In tegenstelling hiermee bleef dit vermogen bij bewaring in diepgevroren suspensies behouden. Bij isolaten van

stam 1 werden geen verliezen in het karakteristieke infectievermogen ten aanzien van *L. esculentum* CStMW-18 en *S. pennellii* waargenomen.

Terwijl stam 0 zeer algemeen in vatbare tomategewassen voorkomt, worden de stammen 1 en 2 minder vaak gevonden. De stam 2^a moet als uiterst zeldzaam worden beschouwd. Daarom mag worden verwacht dat deze stam bij een snel toenemend gebruik van resistente tomaterassen, welke homozygoot zijn voor het gen *Tm*-2^a, niet voldoende tijd zal krijgen zich uit de natuurlijke stammenpopulatie te ontwikkelen.

Tenslotte wordt de praktische toepassing van de symptoomloze nitrietmutant, MII-16, voor opzettelijke kiemplantinfectie besproken. Deze maatregel is gebaseerd op het beginsel van premunitie waarbij stammen van hetzelfde virus elkaar in dezelfde waardplant uitsluiten. Hoewel een infectie met MII-16 enige groeiremming bij tomateplanten veroorzaakt, beschermt hij deze afdoende tegen latere infecties met andere TMV-stammen. In opbrengstproeven is vastgesteld dat een behandeling met MII-16 een optimale opbrengst van de gangbare tomaterassen verzekert. Ook zijn er aanwijzingen dat de mutant gebruikt zou kunnen worden ter verkrijging van praktisch virusvrij zaad. Verder is vastgesteld dat MII-16 een stammenmengsel is bestaande uit de symptoomloze mutant en een op de uitgangsstam gelijkende tomatestam. Bij de laatste vergeleken vermeerdert de mutant zich minder snel en is hij minder goed bestand tegen bewaring in gedroogd blad. Om die redenen is het noodzakelijk de mutant telkens opnieuw uit het stammenmengsel te isoleren en voor praktisch gebruik te leveren in de vorm van gezuiverde suspensies, welke in diepvries bewaard kunnen worden. Het resultaat van een inoculatie met MII-16 is na te gaan door een herinoculatie met een TMV-stam, welke op onbeschermd gebleven planten een geelmozaïek veroorzaakt. Deze methode is te gebruiken zowel bij de ontwikkeling van massa-inoculatietechnieken als bij de controle van smetstof op infectievermogen.

Mozaïeksymptomen welke zich ondanks de toepassing van MII-16 in de praktijk kunnen voordoen zijn te herleiden tot drie oorzaken: een mogelijke verontreiniging van de smetstof, geïnfecteerd zaad en onvolkomenheden bij de uitvoering van de inoculatie. In het algemeen zijn echter met opzettelijke kiemplantinfectie met MII-16 gunstige resultaten bereikt. De ervaringen met MII-16 hebben aangetoond dat van de variabiliteit van het TMV, welke een bedreiging vormt voor de resistente tomaterassen, ook een nuttig gebruik kan worden gemaakt.

References

Ainsworth, G. C., 1933. An investigation of tomato virus diseases of the mosaic 'stripe', streak group. Ann. appl. Biol. 20: 421–428.

Ainsworth, G. C., 1937. 'Enation mosaic' of tomato caused by a virus of the tobacco virus 1 type. Ann. appl. Biol. 24: 545–556.

Ainsworth, G. C., G. H. Berkeley & J. Caldwell, 1934. A comparison of English and Canadian tomato virus diseases. Ann. appl. Biol. 21: 566–580.

Alexander, L. J., 1962. Strains of TMV on tomato in The Netherlands and in Ohio, U.S.A. Meded. LandbHogesch. Opzoekstn Gent, 72: 1020–1030.

Allard, H. A., 1916. The mosaic disease of tomatoes and petunias. Phytopathology 6: 328–335.

Boyle, J. S. & D. C. Wharton, 1957. The experimental reproduction of tomato internal browning by inoculation with strains of tobacco mosaic virus. Phytopathology 47: 199–207.

Broadbent, L., 1961. The epidemiology of tomato mosaic. 1. A review of the literature. Rep. Glasshouse Crops Res. Inst. 1960: 96–116.

Broadbent, L., 1962. The epidemiology of tomato mosaic. 2. Smoking tobacco as a source of virus. Ann. appl. Biol. 50: 461–466.

Broadbent, L., 1964a. Tomato mosaic. N.A.A.S. 9. Rev. 65: 13–22.

Broadbent, L., 1964b. The epidemiology of tomato mosaic. 7. The effect of TMV on tomato fruit yield and quality under glass. Ann. appl. Biol. 54: 209–224.

Broadbent, L., 1965a. The epidemiology of tomato mosaic. 8. Virus infection through tomato roots. Ann. appl. Biol. 55: 57–66.

Broadbent, L., 1965b. The epidemiology of tomato mosaic. 11. Seed-transmission of TMV. Ann. appl. Biol. 56: 177–205.

Broadbent, L., W. H. Read & F. T. Last, 1965. The epidemiology of tomato mosaic. 10. Persistence of TMV-infected debris in soil and the effects of soil partial sterilization. Ann. appl. Biol. 55: 471–483.

Cirulli, M. & L. J. Alexander, 1969. Influence of temperature and strain of tobacco mosaic virus on resistance in a tomato breeding line derived from *Lycopersicon peruvianum*. Phytopathology 59: 1287–1297.

Doolittle, S. P., 1954. The use of wild Lycopersicon species for tomato disease control. Phytopathology 44: 409–414.

Doolittle, S. P. & F. S. Beecher, 1942. A strain of tobacco mosaic virus causing a necrosis and shriveling of tomato foliage. Phytopathology 32: 986–994.

Fletcher, J. T., 1968. Tomato: The effect of tobacco mosaic virus infection on yield and quality. Fourth and fifth year progress reports 1966 and 1967. Rep. Lee Vall. Hort. Stn 1967–'68, p. 74–82.

Fletcher, J. T., 1969. Studies on the overwintering of tomato mosaic in root debris. Pl. Path. 18: 97–108.

Fletcher, J. T. & B. H. MacNeill, 1971. The identification of strains of tobacco mosaic virus from tomato crops in Southern Ontario. Can. J. Microbiol. 17: 123–128.

Friedrich-Freksa, H., G. Melchers & G. Schramm, 1946. Biologischer, chemischer und serologischer Vergleich zweier Parallelmutanten phytopathogener Viren mit Ihren Ausgangsformen. Biol. Zbl. 65: 187–222.

Fulton, R. W., 1941. The behaviour of certain viruses in plant roots. Phytopathology 31: 575–598.

Henderson Smith, J., 1928a. Experiments with a mosaic disease of tomato. Ann. appl. Biol. 15: 155–167.
Henderson Smith, J., 1928b. The transmission of potato mosaic to tomato. Ann. appl. Biol. 15: 518–528.
Hollings, M., 1959 *Nicotiana clevelandii* Gray as a test plant for plant viruses. Pl. Path. 8: 133–137.
Holmes, F. O., 1928. Accuracy in quantitative work with TMV. Bot. Gaz. 86: 66–81.
Holmes, F. O., 1934. A masked strain of tobacco mosaic virus. Phytopathology 24: 845–873.
Holmes, F. O., 1950. Internal-browning disease of tomato caused by strains of tobacco mosaic virus from Plantago. Phytopathology 40: 487–492.
Howles, R., 1961. Inactivation of tomato mosaic virus in tomato seeds. Pl. Path. 10: 160–161.
Jaeger, S., 1966. Milch als Infektionshemmstoff mechanisch übertragbarer Viren in Tomaten- und Gurkenkulturen. Phytopath. Z. 56: 340–352.
Jarrett, P. H., 1930. Streak – a virus disease of tomatoes. Ann. appl. Biol. 17: 248–259.
Jensen, J. H., 1933. Isolation of yellow-mosaic viruses, from plants infected with tobacco mosaic. Phytopathology 23: 964–974.
Jensen, J. H., 1937. Studies on representative strains of tobacco mosaic virus. Phytopathology 27: 69–84.
Jensen, M. H., 1968. Cross protection as a means of reducing severity of TMV in greenhouse tomatoes. Diss. Abstr. Sect. B, 29, 3991-B.
Jockusch, H., 1964. In vivo – und in vitro – Verhalten temperatursensitiver Mutanten des Tabakmosaikvirus. Z. Vererblehre 95: 379–382.
Johnson, J., 1947. Virus attenuation and the separation of strains by specific hosts. Phytopathology 37: 822–837.
Kassanis, B. & I. W. Selman, 1947. Variation in the reaction of White Burley tobacco to the tomato aucuba mosaic virus and some other strains of tobacco mosaic virus. J. Pomol. 23: 167–170.
Kassanis, B. & R. D. Woods, 1969. Properties of some defective strains of tobacco mosiac virus and their behaviour as affected by inhibitors during storage in sap. Ann. appl. Biol. 64: 213–224.
Komochi, S. T., T. Goto & N. Oshima, 1966. Studies on the control of plant virus diseases by vaccination of attenuated virus. 3. Reduction in fruit setting as a shock reaction resulting from infection of tomato plants with TMV. (Jap., English summ.). J. hort. Ass. Japan 35: 269–276.
Komuro, Y., 1963. On the causal viruses of tomato streak diseases in Japan. (Jap., Engl. summ.) Ann. Phytopath. Soc. Japan 28: 40–47.
Komuro, Y. & M. Iwaki, 1968. Strains of tobacco mosaic virus contained in cigarettes, with special reference to the infection source for mosaic disease of tomato. (Jap., Engl. summ.) Ann. Phytopath. Soc. Japan 34: 98–102.
Komuro, Y., M. Iwaki & M. Nakahara, 1966. Viruses isolated from tomato plants showing mosaic and/or streak in Japan, with special reference to tomato strain of tobacco mosaic virus. (Jap., Engl. summ.) Ann. Phytopath. Soc. Japan 32: 130–137.
Kunkel, L. O., 1934. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopathology 24: 437–466.
Laterrot, H. &. P. Pécaut, 1965. La production de semences de tomate. Ann. Epiphyties 16 (hors séries 1): 163–170.
Laterrot, H. & P. Pécaut, 1968. Incidence du traitement thermique des semences de tomate sur la transmission du virus mosaique du tabac. Ann. Epiphyties 19 (hors séries): 159–164.
L.E.I. – informatie. Vroege stookteelten Westland. Groenten en fruit 29: 185.
Lijst van plantenziektenkundige termen, 1968. Neth. J. Pl. Path. 74: 65–84.
MacNeill, B. H., 1962. A specialized tomato form of the tobacco mosaic virus in Canada. Can. J. Bot. 40: 49–51.
MacNeill, B. H. & H. Ismen, 1960. Studies on the virus-streak syndrome in tomatoes. Can. J. Bot. 38: 277–282.
Marrou, J. & A. Migliori, 1965. Méthode d'inoculation rapide, pour l'étude de la resistance. Annls Épiphyt. 16 (hors séries 1): 119–128.

McKinney, H. H., 1929. Mosaic diseases in the Canary Islands, West Africa and Gibraltar. J. agric. Res. 39: 557–558.
McRitchie, J. J. & L. J. Alexander, 1957. Effect of strains of tobacco mosaic on yields of certain tomato varieties. Phytopathology 47: 24 (Abstr.).
McRitchie, J. J. & L. J. Alexander, 1963. Host-specific *Lycopersicon* strains of tobacco mosaic virus. Phytopathology 53: 394–398.
Messiaen, C. M. & P. Maisson, 1962. La mosaique de tabac sur tomate. Annls Épiphyt. 13: 23–38.
Mosch, W. H. M., H. Huttinga & A. Th. B. Rast, 1973. Some chemical and physical properties of 18 tobacco mosaic virus isolates from tomato. Neth. J. Pl. Path. 79: 104–111.
Mundry, K. W. & A. Gierer, 1958. Die Erzeugung von Mutationen des Tabakmosaikvirus durch chemische Behandlung seiner Nucleïnsäure in vitro. Z. Vererblehre 89: 614–630.
Nederpel, jr., L., 1971. Methoden van grondstomen 1969–1970. Intern rapport. Proefstn Groenten- en Fruitteelt Glas, Naaldwijk.
Nederpel, jr., L., 1971. Sterilisatie en pasteurisatie van kasgrond 1970. Intern rapport Proefstn Groenten- en Fruitteelt Glas, Naaldwijk.
Norval, I. P., 1938. Derivatives from an unusual strain of tobacco mosaic virus. Phytopathology 28: 675–692.
Paludan, N., 1968. Tobacco mosaic virus (TMV). Investigation concerning TMV in different plant genera, the virulence of TMV strains, virus attenuation by heat treatment, cross protection and yield (Danish, English summ.). Tidsskr. PlAvl 72: 69–80.
Pécaut, P. & H. Laterrot, 1963. Traitement par la chaleur sèches de graines de tomate: Effet sur la teneur en virus de la mosaique du tabac. INRA Bull.Stn. Amélior. Plantes maraîchères, Montfavet.
Pelham, J., 1966. Resistance in tomato to tobacco mosaic virus. Euphytica 15: 258–267.
Pelham, J., 1968. TMV resistance. Rep. Glasshouse Crops Res. Inst. 1967, p. 45–48.
Pelham, J., 1972. Strain- genotype interaction of tobacco mosaic virus in tomato. Ann. appl. Biol. 71: 219–228.
Pelham, J., J. T. Fletcher & J. H. Hawkins, 1970. The establishment of a new strain of tobacco mosaic virus resulting from the use of resistant varieties of tomato. Ann. appl. Biol. 65: 293–297.
Price, W. C., 1935. Acquired immunity from cucumber mosaic in zinnia. Phytopathology 25: 776–789.
Rast, A. Th. B., 1965. A yellow ringspot strain of tobacco mosaic virus (TMV) from tomato. Neth. J. Pl. Path. 71: 91–93.
Rast, A. Th. B., 1966. J. versl. Inst plziektenk. Onderz. 1965, p. 88–90.
Rast, A. Th. B., 1967a. Yield of glasshouse tomatoes as affected by strains of tobacco mosaic virus. Neth. J. Pl. Path. 73: 147–156.
Rast, A. Th. B., 1967b. Differences in aggressiveness between TMV-isolates on clones of *Lycopersicum peruvianum*. Neth. J. Pl. Path. 73: 186–189.
Rast, A. Th. B., 1967c. J. versl. Inst plziektenk. Onderz. 1966, p. 102–105.
Rast, A. Th. B., 1968a. Evidence for the presence of the Ohio strain III of tobacco mosaic virus in tomato in the Netherlands. Neth. J. Pl. Path. 74: 234–236.
Rast, A. Th. B., 1968b. J versl. Inst. plziektenk. Onderz. 1967: 102–107.
Rast, A. Th. B., 1969. J versl. Inst. plziektenk. Onderz. 1968: 100–102.
Rast, A. Th. B., 1970. J versl. Inst. plziektenk. Onderz. 1969: 106–108.
Rast, A. Th. B., 1972. MII-16, an artificial symptomless mutant of tobacco mosaic virus for seedling inoculation of tomato crops. Neth. J. Pl. Path. 78: 110–112.
Rast, A. Th. B., 1973. Systemic infection of tomato plants with tobacco mosaic virus following inoculation of seedling roots. Neth. J. Pl. Path. 79: 5–8.
Rees, A. R., 1970. Effect of heat treatment for virus attenuation on tomato seed viability. J. hort. Sci. 45: 33–40.
Robb, S. M., 1964. The movement of tomato aucuba mosaic virus in tomato. New Phytol. 63: 267–273.
Roberts, F. M., 1950. The infection of plants by viruses through roots. Ann. appl. Biol. 37: 385–396.
Robinson, R. A., 1969. Disease resistance terminology. Rev. appl. Mycol. 48: 593–606.

Salaman, R. N., 1933. Protective inoculation against a plant virus. Nature 131: 468.
Samson, R. W., 1942. Single virus streak of greenhouse tomatoes. Pl. Dis. Reptr 26: 361–364.
Sehgal, O. P., 1967. Inactivating and mutagenic action of nitrous acid on tobacco mosaic virus and its isolated nucleic acid. Abstr. Phytopathology 57: 830.
Selman, I. W., 1941. The effects of certain mosaic-inducing viruses under glass. J. Pomol. 19: 107–136.
Smilde, K. W. & J. P. N. L. Roorda van Eysinga, 1968. Nutritional diseases in glasshouse tomatoes. Pudoc, Wageningen, 48 pp.
Smith, K. M., 1957. A textbook of plant virus diseases. 2nd ed. J. & A. Churchill, London, p. 505–524.
Smith, P. R., L. L. Stubbs & J. L. Sutherland, 1965. Internal browning of tomato fruit induced by a late infection of tobacco mosaic virus. Aust. J. exp. Agric. Anim. Husb. 5: 75–79.
Steepy, T. L., 1968. Studies involving two tomato virus strains of tobacco mosaic virus (TMV) in tomato *Lycopersicum esculentum* L. 'Revermun'. Tidsskr. PlAvl 72: 81–85.
Taylor, R. H., R. G. Grogan & K. A. Kimble, 1961. Transmission of tobacco mosaic virus in tomato seed. Phytopathology 51: 837–842.
Termohlen, G. & H. J. M. van Dorst, 1959. Verschillen tussen *Lycopersicum* virus 1 en *Nicotiana* virus 1. Jversl. Proefstn Groenten- en Fruitteelt Glas, Naaldwijk 1958: 125–126.
Tomaru, K., K. Suyama & S. Kubo, 1970. Efficiency of local lesion formation and multiplication of tobacco mosaic virus strains in the inoculated leaves of some host plants. (Jap., Engl. summ.) Ann. Phytopath. Soc. Japan 36: 74–80.
Thung, T. H., 1931. Smetstof en plantencel bij enkele virusziekten van de tabaksplant. Handel. 6e Congr. Ned. Ind. Natuurwetensch., p. 450–463.
Van Dorst, H. J. M., 1963. Strepenziekte bij tomaat. Jversl. Proefstn Groenten- en Fruitteelt Glas, Naaldwijk 1962, p. 143–144.
Van Dorst, H. J. M., 1970. Glasshouse Crops Res. Exp. Stn Naaldwijk Ann. Rep. 1969, p. 75.
Van Koot, Y., 1939. De belangrijkste virusziekten van de tomaat in Nederland. Meded. TuinbVoorl-Dienst 10.
Vanterpool, T. C., 1926. Streak or winter blight of tomato in Quebec. Phytopathology 16: 311–331.
Van Winckel, A., 1965. Tabakmozaïekvirus op tomatenzaad. Agricultura, Leuven 4: 721–729.
Van Winckel, A., 1967. Bijdrage tot de studie van het tabakmozaïekvirus (TMV) bij tomaten. Thesis Kath. Univ. Leuven, Fac. Landbouwwetensch., 120 pp.
Van Winckel, A. & A. M. Geypens, 1965. Inactivering van tabakmozaiekvirus in de grond. Parasitica 11: 124–137.
Venekamp, J. H. & W. H. M. Mosch, 1964. Chromatographic studies on plant viruses. 3. The purification of potato virus X, potato virus Y, tobacco mosaic virus by chromatography on cellulose columns with polyethylene glycol containing solutions as solvents. Virology 23: 394–402.
Venekamp, J. H., W. H. M. Mosch & V. Taborsky, 1973. Purification of potato virus X, white clover mosaic virus, tobacco mosaic virus and ribosomes by column chromatography. J. Chromatography 75: 235–246.
Westerdijk, J., 1910. Die Mosaikkrankheit der Tomaten. Med. Phytopath. Lab. 'Willie Commelin Scholten', Baarn 1.
Wheeler, G. F. C., 1961. Glasshouse crops in soilless culture. Comm. Grower 3436: 920–921.
Wlassow, J. I., 1962. Die Bekämpfung von Viruskrankheiten bei Gemüse und Kartoffeln. NachrBl.dt. PflSchutzdienst, Berl. 16: 31–32.

Appendix

Mass purification and testing procedures of MII-16 for use in commercial tomato crops

As the results of cross protection of tomato crops with MII-16 depend largely on the quality of the inoculum used its preparation requires great care.

The tomato plants intended for the extraction of MII-16 are raised from virus-free seeds of the cultivar Moneydor. The plants are inoculated in the seedling stage either individually with single lesions produced on *N. glutinosa* or as a batch. In the former case each of about 200 lesions is used to inoculate a young tobacco plant of the cultivar 'Samsun' in addition to a tomato seedling. Corresponding pairs of plants are discarded if either one or both develop distinct symptoms. Four weeks after inoculation one or two tomato plants are selected to start a new series of single lesions and are then harvested together with the remainder of the plants. A similarly treated mixed population of plants provides the inoculum for mass inoculation of tomato seedlings. This is done most easily by spraying the seedlings in the seed tray one day before they are pricked out. Grown in batches of 600 these tomato plants are carefully examined for symptoms during four weeks and then harvested for extraction. However, the whole batch is discarded if more than five percent of the plants has shown normal mosaic symptoms.

The procedure followed in producing inoculum of MII-16 is essentially a modification of the column chromatographic purification method by Venekamp et al. (1973). For the preparation of crude sap entire infected plants are first minced with a Hobart cutting machine and then ground in a Sorvall omnimixer in portions of 500 g each to which 250 ml of 0.004 M phosphate buffer pH 7 is added. The ground plant material is filtered through an ordinary household plastic sieve. What is left on the sieve is wrapped in two layers of cheese cloth to be pressed in a Hafico hydraulic oil press. The sap as collected under the sieve and the oil press is stirred vigorously for about three minutes in the omni-mixer in amounts of 1000 ml to each of which 25 ml of carbonatetrachloride has been added. The sap is then clarified by centrifugation at 6000 rev./min for 10 minutes in a Sorvall RC-2-B centrifuge. To the brown supernatant thus obtained polyethylene glycol 6000, NaCl, glucose and $MgCl_2$ are added to give final concentrations of 5%, 2%, 4.5% and 0.004 M respectively. This suspension is then ready for transfer to cellulose columns.

For the preparation of a column 20 g of ordinary filter paper previously cut into pieces of 1 cm^2 is ground with a Heidolph Schwabach electric stirrer in 700 ml of Solvent 1, which contains 5% PEG, 2% NaCl, 4.5% glucose, 0.004 M $MgCl_2$ and 0.01 M phosphate buffer pH 7. Shortly before the stirrer is stopped 10 g of cellulose powder (Whatman CF 11) is mixed with the pulp which is then poured into a 1-l

plastic container with a cotton plug at the bottom. The excess of Solvent 1 is drained until the filter paper-cellulose mixture representing the column is compacted into a quarter of the volume of the plastic container. The upper surface of the column is covered with a layer of cotton and a piece of filter paper to break the impact of the liquids which are poured on top.

Starting from 1000 g of infected plant material the brown virus containing suspension obtained is distributed among four of such columns. The rate of flow for each column is adjusted to give 1 – 2 drops per second. The elution of this suspension is followed by a wash with 600 – 800 ml of Solvent 1 for each column. When the liquid dripping from the columns has become colourless they are further eluted with 400 – 600 ml of Solvent 2, which has the same composition as Solvent 1 except that NaCl has been omitted. To the opalescent to milky white virus containing suspension eluted from the columns NaCl is added to give a final concentration of 2%. This is then centrifuged at 10,000 rev/min for 15 minutes and the resulting pellets resuspended in 200 – 250 ml of 0.04 M phosphate buffer. Most recently the suspension has been concentrated so as to give an optical density reading of 0.3 – 0.4 in a 1:10 dilution in a Kipp BFK photometer with a fixed wave length of 254 nm.

The quality requirements of the purified suspension of MII–16 with regard to contamination by the tomato strain as well as infectivity are tested by spray-inoculating a batch of 100 tomato seedlings with a $1:10^3$ dilution. The aim is to produce suspensions which cause not more than one percent of the plants to develop mosaic symptoms within three to four weeks after inoculation. These limitations are rather arbitrary for the number of plants with mosaic symptoms may increase with the number of times they have been hit during inoculation or with the amount of abrasive used in the inoculum. It is also conceivable that the observational period is too short for contaminating strains to become apparent. The infectivity of the suspension is assessed by a challenge inoculation of the tomato plants with the isolate GPga of the yellow mosaic strain which is applied by hand one week after the protective spray inoculation. The plants which are not protected by the latter treatment are identified by the development of the striking symptoms characteristic for the isolate GPga. This isolate is particularly suitable for the purpose as it does not require a waiting time of 10 days between protective and challenge inoculations as do most other isolates. The result of the challenge inoculations with GPga is considered satisfactory if yellow mosaic symptoms appear on less than 10 percent of the plants.

The infectivity of the suspension is further tested by applying it in a series of dilutions on *N. tabacum* 'Xanthi nc'. Although the suspension is meant to be used commercially in a $1:10^3$ dilution the assay on 'Xanthi nc' should indicate a distinct decline in the number of lesions only between $1:10^4$ and $1:10^5$. It should be noted that greater significance is attached to this assay than to the optical density readings.

The suspension is kept in a refrigerator at 2°C for the duration of the tests. After completion of the tests it is distributed in vials in quantities of 5 ml for storage in a deep-freeze box at −18°C.