Host-Pathogen Dynamics of Tobacco Mosaic Virus on Tomato

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Virus disease control in agricultural crops in the past has been dependent on many practices, such as virus-free seed and clones, insect vector control, sanitary measures, heat treatment, and meristem culture. More recently, virus-resistance breeding programs have resulted in the development of many crop plants resistant to one or more virus diseases. A few of the crops with resistance to some virus diseases are corn, sugarcane, wheat, pea, cucumber, sugarbeet, and bean. Although the entire list is too long to enumerate, it is not meant to imply that all virus diseases have been brought under control by host resistance.

Problems facing workers breeding for virus resistance are many. Perhaps the most important of these is locating suitable sources of resistance. Should we look for a nonspecific type of resistance that would be active against many strains of the pathogen, or a specific type of resistance? The latter has been found in tomato and will be elucidated here in more detail. Problems of virus strains often are vexing, and lead to long, drawn-out research programs. This has been particularly true with sugarcane, sugarbeet, tomato, bean, and some other crops.

The gene relationship in the host, especially number of genes, conditions under which they are effective, etc., has caused many problems. However, some cases have been relatively simple when all the factors are known. This is the case with tomato, where several genes for resistance, the manner of inheritance of resistance to the strains, and the environmental conditions under which the genes are operative are all known. However, with multiple genes for resistance and multiple strains of the virus which react differently under varying environmental conditions, it frequently becomes difficult to determine mechanisms of inheritance.

It is not within the scope of this paper to review all literature on the effects of gene relations of hosts to strains of virus and their reaction under various environmental conditions. Rather, I have chosen to illustrate such relationships as have been found in tomato, and refer only to other crops where pertinent. For more detailed literature reviews, readers are referred to the work of Pelham (26) and Walter (38).

Strains of virus.—Problems caused by virus strains are acute because in almost all cases where virus-disease breeding programs have been attempted, pathogenic strains of the virus have been discovered. Such was the case with the tomato breeding program for tobacco mosaic virus (TMV) resistance. Origin of these strains is not understood. Certainly at one time or another they arose by mutation, but did the mutations occur as a response of selection pressure brought about by TMV-resistance breeding programs, or were the strains present from the onset, and only became evident when host genes for resistance made differential stocks available to sort them out? One could reason logically, I think, that the latter primarily occurred, although both may well have been operative.

Symptomatological strains of TMV have been known for many years. However, only recently have specific strains been recognized whose pathogenicity is related to specific genes in the host. It is also known that TMV of tobacco (24) and of tomato (32), as a general rule, are different. Termohlen (37) described two selections of White Burley tobacco, one of which developed a systemic infection when inoculated with TMV from tobacco; the other developed necrotic local lesions when inoculated with TMV from tomato. Thus, he differentiated two strains of TMV, tobacco mosaic virus and tomato mosaic virus. He found also that most of the glasshouse tomatoes of Holland were infected with the tomato strain. Likewise, Broadbent (9) and Broadbent & Fletcher (10) found that glasshouse tomatoes in England were predominantly infected with the tomato strain of the virus. MacNeill (19), working with *Petunia* sp. as an indicator host, found that specialized forms of the virus infected tomatoes in Canada, and the tobacco strain infected tobacco. On the other hand, Alexander (2), working in Holland, single-lesioned virus isolations from both tobacco and tomato and found mixed infections in both cases. Thus, it seems logical to infer that, even though mass tests indicate that the strains predominantly affecting tomatoes are "specialized" and that "regular" strains affect tobacco, both may be present in both hosts as mixtures. It is not known whether any relationship exists between the strains U1 and U2, described by Siegel & Wildman (31), and those described by McRitchie (20, 21) and McRitchie & Alexander (22, 23). However, it appears that U1 is a tobacco and U2 is a tomato strain, even though both were derived from the same sample. Thus, it would appear that the distinction between tobacco and tomato strains may be only of academic interest in a tomato breeding program.

The work of McRitchie & Alexander (22, 23) and Alexander (3) added a new dimension to the TMV strain complex when they distinguished four strains of TMV which affect Ohio-grown tomatoes by the use of differential tomato hosts. Later, Alexander & Cirulli (5), Alexander (4), and Cirulli & Alexander (11) described a fifth strain. These were the first described strains of the virus which infected tomato but which

could be differentiated by specific host varieties in which the gene or genes governing resistance were known. Strains I and II reacted as tobacco and the others as tomato strains of TMV.

A large number of chemical analyses have been made on the protein of TMV strains. Perhaps the work of Wang & Knight (39) is the most complete. They determined the protein components of 13 strains of TMV isolated from tomato from various parts of the world, and found a clear relationship among the strains in amino acid composition and sequence and in serological reactions. Of noteworthy significance was the fact that all strains analyzed, including regular TMV and the tomato strains, possessed 158 amino acid residues. Despite other minor variations, the presence of one methionine residue/protein subunit and C-terminal serine instead of threonine apparently characterized the tomato strains. However, these authors stated that "the nature of the protein coat of a strain has little to do with its ability to infect tomato or any other host. It seems more likely at present that natural selections of strains may be decided on the basis of some fundamental feature of structure of the viral nucleic acid". They then stated, "such distinctive features are now being sought". Actually, Wang & Knight used serology to check strains of their virus isolates. This widely used method is highly regarded as a reliable tool for virus identification. For the most part, if not entirely, the serological reaction is dependent on the protein fraction. Thus, in practice we use the protein coat as an indicator of the nucleic acid composition. This, of course, presupposes that the nucleic acid of each virus and each strain of virus codes its own specific protein coat. Even though the virus protein gives clues that the nucleic acid of various viruses and different strains of virus differ, we still do not know what changes in the nucleic acid actually occur.

The finding of specific genes in tomato for resistance to specific strains of TMV may contribute to the solving of the problem of nucleic acid variation.

Genes for resistance.—The genus *Lycopersicon* is composed of six well-defined species. Since the domestic species of tomato is largely self-fertilized, many of the genes that confer disease resistance probably have been lost through time. Thus, it is necessary to look for resistance in the wild species. There are three well-defined genes for resistance to the tobacco mosaic virus. None, however, confers a hypersensitive reaction comparable to that transferred from *Nicotiana glutinosa* to *N. tabacum* by Holmes (15).

Two genes for TMV resistance in tomato have been recovered from breeding material released by Kikuta & Frazier (17) and Frazier & Dennett (14). This material involved complex crosses among *Lycopersicon esculentum, L. peruvianum, L. pimpinellifolium, L. hirsutum,* and *L. chilense.* Thus, it is only possible to speculate from which parent each gene was obtained. From Frazier's material, Holmes (16) developed pure breeding lines for TMV resistance controlled by one dominant gene. Clayberg et al. (12) assigned the gene symbol *Tm* to this gene. The gene *Tm* confers a high degree of resistance to Ohio strain I and a tendency to escape infection from Ohio strains II and IV.

The second dominant gene was isolated from Frazier's material by Soost (34, 35, 36). Clayberg et al. (12) assigned the symbol *Tm-2* to this gene for TMV resistance. This gene confers resistance to several strains of TMV. One of Pelham's accessions possessing this gene was susceptible in our laboratory when inoculated with Ohio strains IV and V. Similarly, von der Pahlen (25), working in Brazil, found that one of his TMV strains induced a susceptible reaction on plants possessing this gene. Thus, the gene does not confer resistance to all known TMV strains.

Another limitation on the use of this gene is its linkage to the deleterious recessive gene netted virescence, *nv*, which causes stunting and yellowing when homozygous. Originally it was reported that Smith had broken this linkage, but later work by Smith (33) indicated that it had not been broken. Davis & Webb (13) again reported that the linkage may have been broken, but Schroeder & Provvidenti (28) published evidence that Davis & Webb (13) had worked with the gene *Tm,* not *Tm-2.* Recently, Laterrot & Pecaut (18) identified another source of the *Tm-2* gene in *L. peruvianum,* P.I. 126926, without linkage to the deleterious gene, *nv.*

McRitchie & Alexander (22, 23), isolated a third gene for TMV resistance from *L. peruvianum,* P.I. 128650, which was assigned the symbol $Tm\text{-}2^2$ by Schroeder et al. (30). However, Robinson (*personal communication*) pointed out that, according to the rules of gene nomenclature for tomato (7), it would have been more proper to assign the symbol $Tm\text{-}2^a$. Thus, the gene will be referred to here as $Tm\text{-}2^a$. The genes *Tm-2* and $Tm\text{-}2^a$ appear to be at the same locus on chromosome 9.

Because of the interesting intricacies of responses of the $Tm\text{-}2^a$ gene, I would like to detail the results of our studies. By the use of embryo culture, Alexander (1) produced the F_1 interspecific hybrid, *L. esculentum* by *L. peruvianum.* Embryo culture was necessary to obtain the backcross to commercial-type plants, but thereafter, plants of hybrid progenies were fertile. The resulting resistant parent, 801, used in the genetic studies was from seed of an F_4-generation plant, homozygous for resistance. The susceptible parent was an inbred selection of Bonny Best. Reciprocal crosses were made. Since the ratios of resistant to susceptible plants from progenies of the reciprocal crosses were similar, the results were pooled.

In the classification of plants for their reactions to four of the five virus strains, a new class designated "necrotic" was included to indicate heterozygous plants under some conditions. Thus, the three classes, "healthy", "necrotic", and "mottled" were used. "Healthy" indicated symptomless plants. It was not possible to assay all such plants for virus, but many plants selected at random were assayed on *Nicotiana glutinosa* and found to be virus-free. It was assumed that all healthy plants were virus-free. "Necrotic" designated plants developing necrosis after inoculation

TABLE 1. Reaction of F_2 generation tomato plants at two temperature regimes to four strains of tobacco mosaic virus and X^2 tests for goodness of fit (P-value)[a]

F_2 populations	Strains of virus, number of plants, and P-values															
	I[b]				III				V				IV			
	H	N	M[c]	P value	H	N	M	P value	H	N	M	P value	H	N	M	P value
15-16 C																
				(3:1)				(3:1)				(3:1)				(3:1)
(BB × 801)-6[d]	42	0	11	0.50-0.30	45	0	12	0.50-0.30	45	0	20	0.30-0.20	35	0	14	0.70-0.50
(BB × 801)-10	41	0	19	0.30-0.20	42	0	15	0.90-0.80	56	0	11	0.20-0.10	31	0	18	0.10-0.05
(801 × BB)-2	41	0	16	0.70-0.50	38	0	19	0.20-0.10	50	0	17	>0.95	37	0	14	0.70-0.50
(801 × BB)-9	38	0	16	0.50-0.30	45	0	12	0.50-0.30	58	0	15	0.50-0.30	34	0	17	0.20-0.10
27-28 C																
				(1:2:1)				(1:2:1)				(1:2:1)				(3:1)
(BB × 801)-9	12	21	8	0.70-0.50	12	16	13	0.50-0.30	8	24	9	0.70-0.50	23	0	10	0.50-0.30
(BB × 801)-10	9	19	7	0.80-0.70	8	13	12	0.30-0.10	4	18	14	0.10-0.05	24	0	10	0.70-0.50
(801 × BB)-2	8	11	15	0.05-0.02	10	21	9	0.95-0.90	11	15	9	0.70-0.50	23	0	11	0.50-0.30
(801 × BB)-10	13	21	7	0.50-0.30	10	14	10	0.70-0.50	11	15	15	0.20-0.10	28	0	6	0.50-0.30

[a] Control resistant plants, 801, remained healthy; control susceptible plants, BB, were mottled.
[b] Data for strain II are similar to strain I, and thus are omitted.
[c] H = healthy; N = necrotic; M = mottled.
[d] BB = Bonny Best; 801 = homozygous resistant parent.

with strains I, II, III, and V. Necrotic symptoms occurred on heterozygous plants when grown at 27-28 C or slightly higher. "Mottle" designated those plants which developed the well-known symptoms of TMV.

Regardless of temperature regime or strain of virus used, 801 was resistant and Bonny Best (BB) susceptible. At a low temperature regime, 15-16 C, resistance in F_1 plants appeared to be dominant for all five virus strains. But at the high temperature regime, F_1 plants became necrotic when inoculated with strains I, II, III, and V, but not with strain IV.

Reactions obtained when plants of F_2 progenies were inoculated individually with four strains of the virus at the two temperature regimes are shown in Table 1. A total of 924 plants from four F_2 populations was tested for resistance to strains I, III, IV, and V at 15-16 C. In all cases, the plants segregated only for the two classes, healthy and mottled, in a 3:1 ratio. The P-values indicated a reasonably good fit. However, when 452 plants of four similar F_2 progenies were tested individually at the high temperature regime, 27-28 C, against strains I, III, and V, the three classes, resistant, necrotic, and mottled were observed in ratios of 1:2:1, respectively. When 135 different plants of the same four progenies were inoculated with strain IV, only resistant and mottled classes were observed in a 3:1 ratio. Thus, the F_2 data support the hypothesis that the inheritance of resistance to the five Ohio strains of TMV is governed by a single, dominant gene. The data further indicate that at high temperatures heterozygous plants can be distinguished from homozygous resistant plants with the necrotic strains I, II, III, and V, but not with the nonnecrotic strain IV.

To further test the single-dominant-gene hypothesis, backcrosses were made to susceptible and resistant parents. At 15-16 C, a total of 432 plants (Table 2) of two backcross progenies to the susceptible parent, BB, segregated into a ratio of 1 healthy (219) to 1 susceptible (213) when inoculated separately with strains I, III, IV, and V of the virus. However, when 521 plants of four backcross progenies to the susceptible parent were inoculated with strains I, III, and V and tested at 27-28 C, the plants segregated into a ratio of 1 necrotic (282) to 1 mottled (239). Again the results indicated that strains I, III, and V produce necrosis on heterozygous plants at high temperatures. Plants inoculated with strain IV segregated 1 healthy (72) to 1 mottled (91).

All plants of backcrosses to the resistant parent, 801, were resistant at 15-16 C with the four strains of the virus, whereas plants of similar backcrosses tested at 27-28 C segregated into a ratio of 1 resistant (281) to 1 necrotic (253) with strains I, III, or V. Individual P-values indicated a reasonably close fit for 1:1 ratios. All plants inoculated with strain IV remained healthy at both temperature regimes. These and other supporting data of Cirulli & Alexander (11) clearly demonstrate that the gene *Tm-2*[a] is a single dominant gene which confers resistance to the five Ohio strains of TMV. Furthermore, strains I, II, III, and V are of the necrotic type, but differ in that I, II, and III produce a mild necrosis whereas V produces a severe necrosis. The data also indicate that it is possible to distinguish heterozygous plants in F_2 or other segregating populations with necrotic strains of TMV. Since some strains of the virus produce necrotic symptoms at high temperatures, it could be inferred that there is incomplete penetrance of the *Tm-2*[a] gene.

In this work, it also was shown that some homozygous resistant plants can be infected. The conditions under which such infection occurs are not completely understood. However, it may be related to inoculum dosage, temperature regime, and virus strain. Schroeder et al. (30) studied the effects of five different temperature regimes, 15, 25, 30, 35, and 40 C on symptom

TABLE 2. Reaction of backcross tomato plants at two temperature regimes to five strains of tobacco mosaic virus and X^2 tests for goodness of fit (*P*-value)

	Strains of virus, number of plants and P-values															
	I				III				V				IV			
Backcrosses	H	N	M[b]	*P* value	H	N	M	*P* value	H	N	M	*P* value	H	N	M	*P* value
								15-16 C								
Backcross to susceptible parent				(1:1)				(1:1)				(1:1)				(1:1)
(801 × BB)-1 × BB[a]	21	0	26	0.50-0.30	23	0	17	0.50-0.30	32	0	26	0.50-0.30	22	0	28	0.50-0.30
(BB × 801)-4 × BB	34	0	23	0.20-0.10	25	0	26	0.90-0.80	36	0	34	0.90-0.80	26	0	33	0.50-0.30
Backcross to resistant parent																
(801 × BB)-7 × 801	56	0	0		49	0	0		68	0	0		58	0	0	
(BB × 801)-10 × 801	54	0	0		55	0	0		69	0	0		50	0	0	
								27-28 C								
Backcross to susceptible parent																
(801 × BB)-1 × BB	0	26	13	0.05-0.02	0	21	20	0.90-0.80	0	23	18	0.50-0.30	17	0	16	0.90-0.80
(801 × BB)-4 × BB	0	21	27	0.50-0.30	0	24	13	0.10-0.05	0	22	25	0.70-0.50	20	0	28	0.30-0.20
(BB × 801)-1 × BB	0	28	21	0.50-0.30	0	31	17	0.05-0.02	0	23	25	0.80-0.70	21	0	27	0.50-0.30
(BB × 801)-4 × BB	0	20	21	0.90-0.80	0	21	20	0.90-0.80	0	22	19	0.70-0.50	14	0	20	0.50-0.30
Backcross to resistant parent																
(801 × BB)-7 × 801	14	27	0	0.05-0.02	22	19	0	0.70-0.50	13	28	0	0.02-0.01	34	0	0	
(BB × 801)-4 × 801	22	19	0	0.70-0.50	21	20	0	0.90-0.80	20	20	0	0.99	33	0	0	
(BB × 801)-7 × 801	29	20	0	0.20-0.10	30	18	0	0.10-0.05	27	21	0	0.50-0.30	47	0	0	
(BB × 801)-7 × 801	26	23	0	0.70-0.50	34	14	0	<0.01	23	24	0	0.90-0.80	48	0	0	

[a] BB = Bonny Best; 801 = homozygous resistant parent.
[b] H = healthy; N = necrotic; M = mottled.

expression following inoculation of tomato plants with strains of TMV which in some respects resembled Ohio strains IV and V. Homozygous progenies resistant for the gene *Tm-2*[a] inoculated with strain IV produced necrosis in all plants at 35 and 40 C, and with strain V produced necrosis at 35 C. In other cases plants were symptomless. Assays of symptomless plants on *Chenopodium quinoa,* reported to be more sensitive to TMV than *Nicotiana glutinosa,* revealed that all were infected with virus. The length of time between inoculation and assay, the part of the plant assayed, and the amount of virus present were not specified. Thus, it is difficult to estimate the significance of the results. On the other hand, they stated that, "In most instances, inoculations were made once on the cotyledons and the first true leaf with either a glass spatula or cotton swab. A second inoculation was made in some experiments on the stem and the second true leaf when it became fully expanded." This raises the question of the validity of heavy doses of inoculum and repeated inoculations. If under natural conditions, plants are exposed to only limited amounts of inoculum, then should we in our breeding work for virus resistance use extreme methods of testing? For my part, I believe that it is necessary to determine the effects of inoculum dosage on test plants first, then proceed with a differential method that perhaps might be midway between extreme and limited inoculations. Furthermore, it is conceivable that repeated heavy inoculations would obscure the effects of single gene types of resistance. It is well known that extremes in environment, such as temperature and the presence of other pathogens, etc., can obscure simple gene ratios. A case in point is the well-known effect of high temperature on negating the effects of the single dominant gene in cabbage for resistance to yellows caused by *Fusarium oxysporum* f. sp. *conglutinans.*

Alexander (2) and Alexander et al. (6) showed that plants of *L. peruvianum,* P.I. 128650, can be infected by grafting (Table 3). The symptoms of such infected plants are leaf necrosis, stunting of plants, and little fruit. However, the virus concentration in such plants is very low. Cirulli & Alexander (11) further showed that the same reaction occurred when they grafted plants of the homozygous resistant 801 to infected Bonny Best plants, and also that the virus concentration was low. The question still unresolved is how much of the virus in such infected plants is actually produced there and how much is transported from the infected susceptible stock or scion. The symptoms produced by the low virus concentration on such plants are disproportionately severe in contrast to those present in susceptible varieties, such as Bonny Best.

Schroeder et al. (30) stated that the necrotic symptoms in tomato are not reversible with a change from a high to a low temperature. Such may well be the case. However, cuttings made from graft-infected plants in 1965 did make a complete recovery as far as symptoms were concerned when planted in the field for one growing season (Alexander, *unpublished data*). Unfortunately, the plants were not assayed for virus. In a similar experiment in 1968, cuttings from graft-infected plants made no recovery; in fact, some died.

This lack of recovery is in contrast to the effects of

TABLE 3. Graft transmission of TMV from infected Bonny Best (BB) plants to resistant P.I. 128650-6Y-IV-1-12 plants[a]

Graft no.	Stock	Scion	Symptoms on 128650	P.I. 128650 assay results lesions/leaf on *Nicotiana glutinosa* 1st	2nd	3rd
1	128650	BB	Severe mottle	None	3	None
2	128650	BB	Severe mottle	Many	16	100
3	BB	128650	Severe mottle	4	16	25
4	BB	128650	Severe mottle	1	10	25
5	128650	BB	Severe mottle	18	10	
6	128650	BB	Severe mottle	None	3	None
7	BB	128650	Severe mottle	2	9	None
8	BB	128650	Severe mottle	None	4	50
9	BB	128650	Severe mottle	23[b]		
10	BB	128650	Severe mottle			
11	BB	128650	Severe mottle	11[b]		

[a] Assays of infected Bonny Best to *N. glutinosa* always gave too many lesions to count.
[b] Average number of lesions on six *N. glutinosa* leaves.

pea mosaic virus (PMV) in peas [Barton et al. (8) and Schroeder et al. (29)]. Heterozygous pea plants exhibited symptoms when inoculated with pea mosaic virus at high temperatures, but the same plants became symptomless when transferred to low temperatures. Thus, the *mo* gene for resistance to pea mosaic virus differs from the gene *Tm-2*^{a} in tomato in two ways; firstly, the *mo* gene is recessive in contrast to the dominant gene *Tm-2*^{a}; and secondly, the disease symptoms are reversible in the case of the pea virus, but usually not so in tomato.

Gene and strains of virus.—Earlier, the three genes *Tm, Tm-2,* and *Tm-2*^{a}, and the five Ohio strains of TMV were discussed. Pelham (27) proposed that since Ohio strains I and II are similar, they be designated strain O; that Ohio strain III be designated strain I; Ohio strain IV then becomes strain II and Ohio strain V becomes strain III. Whether the combination of Ohio strains I and II is wise is debatable because of the differing responses of lines with *Tm*. With the three genes for resistance, Pelham concluded that eight strains, theoretically, can be identified on a virulent-avirulent basis. However, only four thus far have been found. If this be the case, we should expect to find the other four. The reactions of the eight strains on seven host differentials as visualized are shown in Table 4.

Since the genes *Tm-2* and *Tm-2*^{a} are allelic, the two genotypes containing them must be heterozygous. What effect the finding of three of these additional strains would have on the adequacy of the present breeding program is not known. The finding of theoretical strain VII would necessitate the discovery of at least one new gene.

Pelham (26, 27) has made an urgent plea that no tomato cultivars for TMV resistance be released unless they contain two or more known genes for resistance either as inbreds or F_1 lines. The basis for this reasoning appears sound. That is, if and when a variant of the virus occurs that can overcome the resistance conferred by the gene *Tm-2*^{a}, the variant strain is less likely to be infective if it also has to overcome the resistance imparted by an additional gene or genes. On the other hand, two situations have mitigated against the adoption of this plan in our breeding program in Ohio. Firstly, six undesirable gene associations with *Tm-2*^{a} were found which had to be overcome before desirable commercial tomato varieties could be developed and introduced. These include (i) a tendency for the plants to grow in a horizontal direction, a problem with trellised plants both out-of-doors and in the greenhouse because such plants frequently break when straightened; (ii) a tendency for poor fruit set; (iii)

TABLE 4. Reactions of the four known and expected reactions of four hypothetical strains of TMV to seven host differentials containing five homozygous tomato genotypes and two possible heterozygous combinations

Strains	Genotypes *Tm*	*Tm-2*	*Tm-2*^{a}	*Tm, Tm-2*	*Tm, Tm-2*^{a}	*Tm-2, Tm-2*^{a}	*Tm, Tm-2, Tm-2*^{a}
				Known strains			
O	T[a]	I	I	T-I	T-I	I	T-I
I	S	I	I	S-I	S-I	I	S-I
II	T	S	I	T-S	T-I	S-I	T-S-I
III	S	S	I	S	S-I	I-S	S-I
				Hypothetical strains			
IV	T	I	S	T-I	T-S	I-S	T-I-S
V	S	I	S	S-I	S-S	I-S	I-S
VI	T	S	S	T-S	T-S	S-S	T-S
VII	S	S	S	S	S	S	S

[a] T = Tolerant or tendency to escape infection; I = Immune or resistant; S = Systemic or susceptible.

a tendency for excessive vegetative growth; (iv) small fruit; (v) smooth stem (observed early in the breeding program but considered unimportant; however, when such plants were tried commercially, the workers complained bitterly because they said handling the plants felt like handling snakes); (vi) blotchy ripening (graywall), perhaps the most serious association observed.

The first five undesirable linkages have been overcome satisfactorily, and it now appears that a satisfactory degree of resistance to blotchy ripening (graywall) has been combined with TMV resistance, but the high resistance of the graywall resistant parent, Ohio W-R 25, probably has not been entirely transferred. Nine backcrosses to commercial types have been required. Thus, many plants have been grown and 3 years have elapsed in order to break, or at least partially break, this one association.

Secondly, can we justifiably delay for a period of years the introduction of a TMV-resistant variety with multiple genes? I do not think so, even though it could logically be argued that the breeding program should have had this objective when initiated. However, one should remember that the addition of each new gene included in breeding objectives, even though dominant, adds materially to the number of plants that must be grown. Furthermore, the space and labor that must be added for each additional genotype increases greatly when one is concerned with thousands of plants. Perhaps another pertinent factor in Ohio is that the fresh market and glasshouse growers are hard pressed by competition from other states and Mexico. They seriously need quick relief from the ravages of the most serious disease of their crop.

How long will present genes be effective?—I know of no way to predict the time of occurrence of new pathogenic strains of the virus. The gene *Tm-2*^{a} is being tried currently in many places in the world, and so far there are no reports of a strain or strains of TMV that will attack plants which have the gene. However, I feel, because of experience with variability of biological material, that new strains of the virus will occur. The precedent has been found with fungi, bacteria, and other viruses. If only 5 years elapse between the introduction of a new variety and the discovery of new pathogenic strains of TMV, the cost of the work will have paid for itself many times over. If we can obtain 10 years of relief by the use of the single gene *Tm-2*^{a}, we will have accomplished wonders. Irrespective of how long the resistance of the gene *Tm-2*^{a} is effective, that much time has been gained for tomato production free of TMV and for research workers to improve their genetic materials.

Of theoretical value would be a sudden change from TMV-susceptible to TMV-resistant cultivars because there would be no tomato host upon which the virus could develop and produce new mutations. Such a change may not be feasible, since growers cannot afford suddenly to plant all his acreage to a new relatively untried cultivar, especially when it is well known that such cultivars frequently require different amounts of fertilizer elements, temperature, water, spacing, etc.

With these limitations, it would appear that we shall have to introduce new cultivars when they are ready and hope that new genes for TMV resistance can and will be found when needed.

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