ever, fewer of the plants recover and recovery seems not to be so complete as that reported by both Wingard and Price in their studies of tobacco ring spot.

Tomato plants of a recovered clone are relatively slow-growing and have other mild curly-top symptoms characteristic of regenerated shoots. In spite of this condition they may be fairly productive. In 1937, out of 15 plants from a recovered clone of Guasave A that survived a fairly heavy inoculation in addition to the rather high concentration of virus already present, 10 plants produced a good crop of fruit (Fig. 1) and the remainder a light crop, which matured about the normal time. All the plants were of less than normal size, even allowing for closeness of planting. Re inoculation of such plants in the summer had no noticeable effect on them. Clones from healthy plants of other races inoculated at the same time developed severe curvy-top symptoms and many of them died; the surviving affected plants produced little or no fruit. In 1937, 19 seedlings of Guasave A were inoculated and all of them developed severe curvy top. All of these seedling plants showed regeneration, but recovery was too late for the production of a crop that season.

In some areas of the western United States curvy top is the limiting factor in tomato production. So far, attempts to select or develop resistant commercial varieties have largely failed. No race of tomato, either wild or cultivated, has been found that is highly resistant to initial infection with curvy-top virus. There is some difference in the reaction of certain races or varieties of tomato to curvy top but none have shown sufficient resistance to make them of much practical value. Tomato plants affected with curvy top do, however, sometimes recover, in part, at least, and acquire a tolerance to the virus. The tomato plants that acquired tolerance to curvy top in these studies, with one possible exception, belonged to wild races. Recovery has been observed in cultivated varieties, but, since an intensive study of this problem was begun, sufficient material has not been available to permit conclusions regarding the association of acquired tolerance with recovery in cultivated varieties.

It may be possible to develop by hybridization a desirable tomato, high in recovery from and tolerant to curvy top, and of sufficiently early maturity to enable recovered seedlings to produce a satisfactory crop during the first season’s growth. However, if a clone of a larger-fruited variety than Guasave A can be obtained, with similar tolerance to curvy top, it would be worth while to overwinter it and propagate it by cuttings for use in areas where curvy top seriously limits tomato production.

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INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN TOBACCO

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In 1914, Allard (1) found that the failure of Nicotiana glutinosa L. to show chlorosis after inoculation with tobacco-mosaic virus was shared by the hybrid N. tabacum L. × N. glutinosa. This hybrid has since been found to respond to infection with tobacco-mosaic virus, as N. glutinosa does, by the production of necrotic primary lesions (7, p. 992; 9). In these lesions the virus is localized except in young plants where systemic necrosis frequently occurs. Whether the necrosis is localized or systemic, ordinary contaminative contacts between leaves of infected and healthy individuals are infective in spreading the disease, in contrast to the high infectivity associated with the systemic chlorosis caused by infection with tobacco-mosaic virus in N. tabacum.

In preliminary experiments, designed to introduce into Nicotiana tabacum the necrotic type of response to infection characteristic of N. glutinosa, attempts were made to obtain seed from the first generation hybrid N. tabacum × N. glutinosa. Plants of this hybrid were grown continuously in greenhouse and garden, often in considerable numbers, for more than 3 years. During this period the hybrid proved consistently self-sterile and sterile to all tested pollens. Finally, because of the failure of repeated attempts to
cross it with varieties of *N. tabacum*, a fertile amphidiploid derived from this hybrid was obtained through the kindness of Dr. R. E. Clausen of the University of California. This amphidiploid species, *N. digluta* Clausen and Goodspeed, was found to resemble *N. glutinosa* in its response to infection with tobacco-mosaic virus. It was therefore used in further breeding experiments. The purpose of this paper is to report the segregation of disease types in successive generations.

EXPERIMENTS WITH DERIVATIVES OF NICOTIANA DIGLUTA

The species *Nicotiana digluta*, described by Clausen and Goodspeed (21), is a self-fertile amphidiploid originating from the hybrid *N. glutinosa* (12) × *N. tabacum* (n = 24), has been studied intensively in the past, but not from the point of view of disease resistance. Derivatives of the form *N. digluta* × *N. tabacum* and (*N. digluta × N. tabacum*) × *N. tabacum* were produced and described by Clausen (3). Since they were not tested by inoculation, however, it is not known whether the necrotic type of response was retained in any individuals of this series of hybrids. In such repeated backcrosses, any characteristic dependent on a gene, or genes, introduced from the non-recurrent parent naturally would be eliminated unless specifically demonstrated and retained in each generation.

As a preliminary to the study of disease types in hybrid generations, 75 virus, applied by rubbing. They all produced necrotic local lesions resembling those of *N. glutinosa*. On maturing they proved self-fertile and also set seed readily when encapsulated flowers were treated with pollen from *N. tabacum*.

In the first hybrid generation, *Nicotiana digluta × N. tabacum* var. Connecticut Broadleaf, 132 plants were grown and tested. They all responded to infection with tobacco-mosaic virus by production of necrotic lesions like those of *N. digluta* and *N. glutinosa*.


The results of tests of the first backcross generation, together with those of subsequent backcrossed and selfed generations, are shown in table 1.

<table>
<thead>
<tr>
<th>Number of</th>
<th>Number of</th>
<th>Connecticut</th>
<th>Burley</th>
<th>Samsun</th>
</tr>
</thead>
<tbody>
<tr>
<td>times with</td>
<td>times</td>
<td>Broadleaf</td>
<td>(Burley</td>
<td>selfed</td>
</tr>
<tr>
<td><em>N. tabacum</em></td>
<td>selfed</td>
<td>backcross</td>
<td>14</td>
<td>backcross</td>
</tr>
<tr>
<td>parent</td>
<td>line</td>
<td>line</td>
<td>132:0</td>
<td>273:0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>121:104</td>
<td>269:0</td>
<td>269:6</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>63:185</td>
<td>150:115</td>
<td>129:135</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>324:136</td>
<td>324:136</td>
</tr>
</tbody>
</table>

+ Beginning with the necrotic type hybrid *N. digluta × N. tabacum* variety Connecticut Broadleaf, backcrosses were made to the three varieties of *N. tabacum*, Connecticut Broadleaf, Burley, and Samsun, a necrotic type hybrid of the preceding generation being used as a parent, *N. tabacum* as a parent in each case.

+ Beginning with a necrotic type plant of *B₁*, as origin in each series, a series of six generations was produced, using a necrotic type plant of the preceding generation as parent in each case.

+ Because *N. digluta* itself originally had *N. tabacum* as one parent, and carried a full set of *N. tabacum* chromosomes, the numbers in this column are larger by one than is usually the case for hybrids of the indicated generations.

Introduction from *Nicotiana glutinosa* of a single dominant gene inducing necrotic type response to infection by tobacco-mosaic virus. This gene will be referred to as *N* (necrotic type response to infection with tobacco-mosaic virus), and its recessive allele, characteristic of *N. tabacum* and of chlorotic type derivatives of the *N. digluta-teaacum* cross, will be referred to as *n* (chlorotic type response). Only 2 disease types were observed in the entire series of generations; these were the necrotic and chlorotic types, characteristic of *N. glutinosa* and *N. tabacum*, respectively. No difference was observed between the response of heterozygous (Nn) plants and that of homozygous (NN) plants. No modification of the action of the *N. glutinosa* gene *N* was detected at any time, despite the varied genetic constitutions of the hybrids into which it was introduced.

In the Burley backcross line, segregation of a pair of genes, presumably the genes *G₃* and *G₄* of Henika (5), controlling green vs. white leaf color, was found to be independent of that of the pair of genes *Nn* controlling necrotic vs. chlorotic type of disease. The observed ratios were 71 green necrotic : 69 white necrotic : 70 green chlorotic : 70 white chlorotic, among progeny from doubly heterozygous green necrotic ♀ and doubly recessive white chlorotic ♂ parents.

Throughout this investigation it was found that spread of virus occurred as promptly in the very youngest necrotic type plants as in comparable chlorotic type plants. When early diagnosis of disease type was desired and plants were also to be saved for production of seed, the severity of the sys-
soum plant were found to be consistently of chlorotic type (250 individuals tested). Selfed progeny of the F₁ plant were those reported in the table as the F₁ generation (363 individuals tested, all found to be of necrotic type). In each of the reciprocal crosses, 250 plants of the progeny were tested by inoculation. Without exception these proved to be necrotic-type plants. This constituted a critical test of 250 male and 250 female gametes, all of which were thus found to carry the newly introduced gene N. A single failure among the 500 would have been disclosed by the appearance of a chlorotic-type plant. To have made an equally sensitive test of gametic purity by self-pollination would have required the square of this number, i.e., 250,000 plants, since the detection of 1 failure in 500 gametes would have been possible only if that gamete happened to fertilize, or to be fertilized by, a similarly rare gamete also failing to carry the gene N. The demonstration of gametic purity of the homozygous NN line of Samsoum tobacco is thus very satisfactory.

It is believed that similar homozygous lines corresponding to locally desirable horticultural varieties of Nicotiana tabacum can be produced readily, either by repeated backcrosses of the homozygous stock of Samsoum tobacco described, with desired types as recurrent parents, or by similar crosses of necrotic-type, Nn plants of the F₁ generation described in this paper (see table 1). These F₁ plants possess the advantage of having been crossed repeatedly to N. tabacum varieties, with opportunity for crossing over to have transformed the chromosome that bears the newly introduced gene N into an essentially N. tabacum type of chromosome.

**DISCUSSION**

Type of Resistance Conferred by the Gene N

Immunity from infection by tobacco-mosaic virus is unknown among species of the genus Nicotiana. There are, however, important differences in type of response to infection within the confines of the genus (6). Some Nicotiana species tend to remain symptomless after they are infected; others show systemic chlorosis. Both the symptomless and the chlorotic-type species, once infected, retain the virus throughout their natural span of life, facilitating spread of virus by providing a good source of inoculum. Still other Nicotiana species show systemic necrosis or localized necrosis as a result of infection. The necrotic-type species are protected from plant-to-plant spread of all strains of the virus by early death of invaded tissues, with consequent impairment of most of the virus. Their response constitutes an effective type of resistance to the disease from a practical viewpoint, in the sense that spread of the disease through the population is greatly impeded. This particular kind of resistance occurs also among other solanaceous plants, as, for example, in some varieties of eggplant, Solanum melongena L. Thus, the Black Beauty eggplant dies as a result of systemic necrosis if infected when young, but localizes the virus in necrotic primary lesions if infected when older (6, p. 333). Under field conditions, injury to this necrotic-type eggplant is unknown, apparently because no strain of the virus becomes established in large amounts within the crop. Vallee (12) has referred to this type of resistance as depending on sensitivity with respect to the virus.

In the past there have been no necrotic-type varieties of tobacco, Nicotiana tabacum. All varieties except the symptomless Ambalena tobacco and its derivatives (10, 11) have shown the classical mottling type of systemic chlorosis after infection with ordinary tobacco-mosaic virus. Very large amounts of virus develop in the infected chlorotic-type plants. Consequently, these plants serve during the remainder of their life, and later, when dried, as the principal reservoir from which comes the virus for contagious infection of later crops of tobacco and other susceptible cultivated species. It is to combat this accumulation of virus in the tobacco crop, rather than to protect the individual infected plant, that a necrotic-type response may prove useful if it can be introduced into horticulturally acceptable strains of tobacco.

In the pepper, Capsicum frutescens L., complete localization of tobacco-mosaic virus occurs at ordinary temperatures in all plants bearing a dominant gene L (7, 8). This localization is permanent because the inoculated leaf is lost by abscission soon after the appearance of necrotic lesions at the site of inoculation. The infected individual is adequately protected. This must not be expected in necrotic-type tobacco, for, although all strains of tobacco-mosaic virus elicit the necrotic type of response, leaf abscission does not follow infection. Both the degree of localization of virus that occurs in old plants and the death of young infected plants, however, would be efficacious in preventing the development of large amounts of inoculum in tobacco. The symptomless condition in the variety Ambalena tends to serve the same purpose with less risk to individual infected plants, but is controlled by a more complex genetic system (4), and is believed to be less uniform in its response to different strains of the virus (12, p. 207).

In the course of the present investigation, a dominant gene for necrotic-type response to infection with tobacco-mosaic virus was incorporated in an inbred line of Nicotiana tabacum. This made available a necrotic-type strain of tobacco which is fully fertile with the innumerable tobacco varieties now grown.

The protective gene is inherited as a Mendelian dominant. Its identification by inoculation methods is readily accomplished. It is hoped, therefore, that further work on the incorporation of the gene into locally acceptable strains of tobacco and subsequent trials under field conditions may be left largely to those who are especially interested in the maintenance and improvement of varieties of tobacco.

**Earlier Recognized Functions of the Gene N**

The dominant gene N of Nicotiana glutinosa, with which the present investigation is concerned, has played an important rôle in the study of tobacco-mosaic virus, in part even before its identity was recognized. Al-