

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 18 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

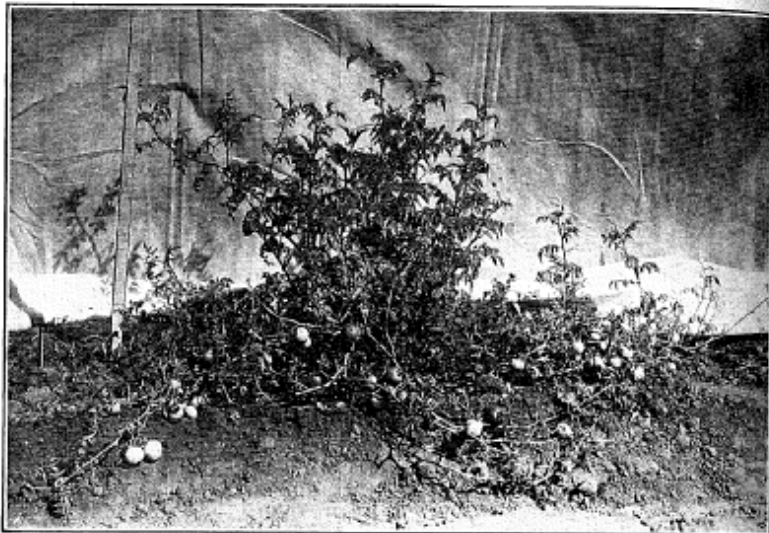


FIG. 1. Acquired tolerance in a tomato plant of a Guasave A clone derived from a seedling that recovered from curly top. The mature fruits are crimson and averaged 0.5 oz. in weight. (Photo. Oct. 11, 1937.)

a light crop, which matured about the normal time. All the plants were of less than normal size, even allowing for closeness of planting. Reinoculation 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 curly-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 curly 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 curly 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

curly-top virus. There is some difference in the reaction of certain races or varieties of tomato to curly top but none have shown sufficient resistance to make them of much practical value. Tomato plants affected with curly top do, however, sometimes recover, in part, at least, and acquire a tolerance to the virus. The tomato plants that acquired tolerance to curly 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 curly 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 curly top, it would be worth while to overwinter it and propagate it by cuttings for use in areas where curly 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. \times *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 ineffective 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* \times *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 (2) as a self-fertile amphidiploid originating from the hybrid *N. glutinosa* ($n=12$) \times *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* \times *N. tabacum* and (*N. digluta* \times *N. tabacum*) \times *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 plants of *Nicotiana digluta* were tested by inoculation with tobacco-mosaic 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 emasculated flowers were treated with pollen from *N. tabacum*.

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

The F_1 plants were crossed with 3 varieties of *Nicotiana tabacum*, Connecticut Broadleaf, White Burley, and Samsoun tobacco. Segregation of the parental disease types occurred among the progeny. Thus (*N. digluta* \times *N. tabacum* var. Connecticut Broadleaf) \times *N. tabacum* var. Connecticut Broadleaf gave a ratio of 121 necrotic-type plants to 504 chlorotic-type plants. (*N. digluta* \times *N. tabacum* var. Connecticut Broadleaf) \times *N. tabacum* var. White Burley gave 272 necrotic-type to 269 chlorotic-type plants. (*N. digluta* \times *N. tabacum* var. Connecticut Broadleaf) \times *N. tabacum* var. Samsoun gave 310 necrotic-type to 357 chlorotic-type plants.

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

Except among the derivatives of Connecticut Broadleaf tobacco, it will be seen from the table that in successive generations the ratios of necrotic-type to chlorotic-type plants, though at times deficient, tended to become the typical monohybrid 1:1 backcross and 3:1 selfed ratios indicative of the

TABLE 1.—Ratios of necrotic-type to chlorotic-type plants among derivatives of *Nicotiana digluta* Clausen and Goodspeed

F_1 hybrid and backcross generations ^a	Number of times with <i>N. tabacum</i> as parent ^b	Connecticut Broadleaf backcross line	Burley (Burley 16) backcross line	Samsoun backcross line
<i>N. digluta</i> \times <i>N. tabacum</i> F_1 ..	2	132: 0
B ₁	3	121: 504	272: 269	310: 357
B ₂	4	17: 247	99: 148	93: 171
B ₃	5	23: 286	123: 120	96: 149
B ₄	6	63: 185	156: 115	120: 135
Selfed generations ^c	Number of times selfed	Connecticut Broadleaf selfed line	Burley selfed line	Samsoun selfed line
F_2	1	310: 144	325: 136	324: 110
F_3	2	271: 182	328: 121	399: 0
F_4	3	123: 79	347: 128	303: 0

^a Beginning with the necrotic-type hybrid *N. digluta* \times *N. tabacum* variety Connecticut Broadleaf, backcrosses were made to the three varieties of *N. tabacum*, Connecticut Broadleaf, Burley, and Samsoun, a necrotic-type hybrid of the preceding generation being used as ♀ parent, *N. tabacum* as ♂ parent in each case.

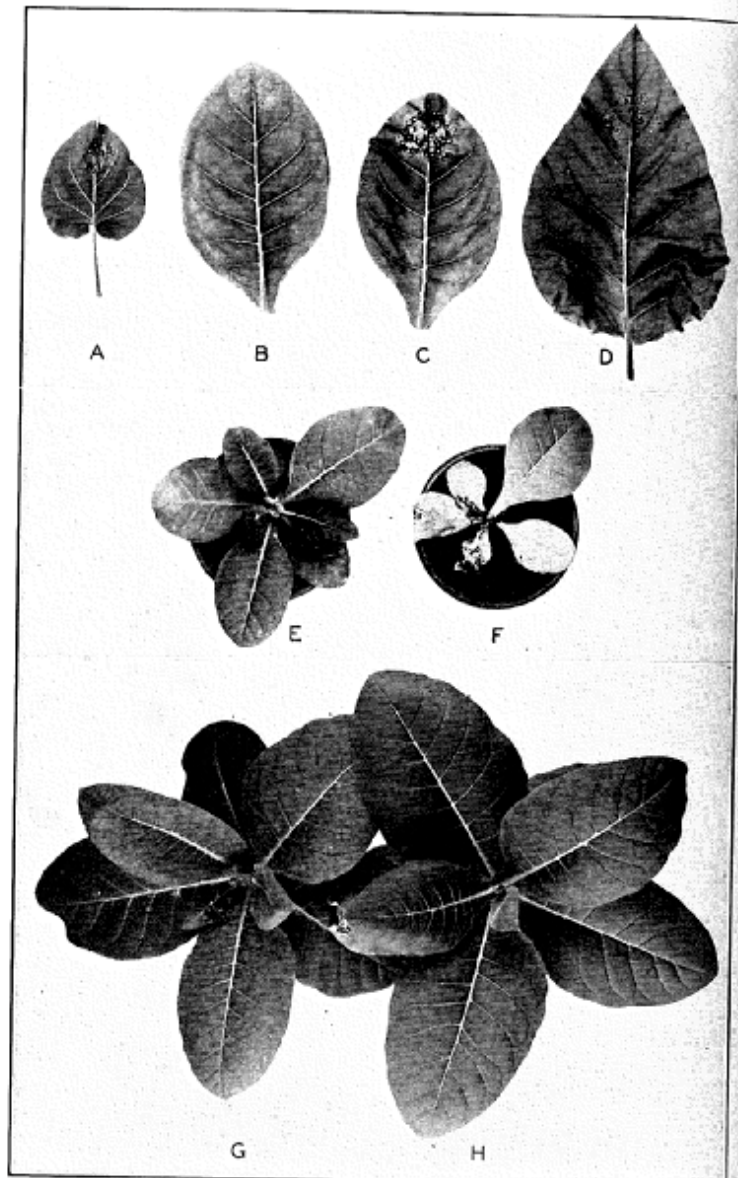
^b Beginning with a necrotic-type plant of B₁ as origin in each series, a series of filial generations was produced, using a necrotic-type plant of the preceding generation as parent in each case.

^c 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*-*tabacum* 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 *G*₁*g*₁ or *G*₂*g*₂ 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 about 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-



Photographs by J. A. Carlile.

FIG. 1. Leaves 5 days after inoculation with distorting-strain tobacco-mosaic virus, and plants 14 days after similar inoculation. A. From NN plant of *Nicotiana glutinosa*. B. From nn plant of *N. tabacum*, Burley backcross. C. From Nn plant of same. D. From NN plant of the repeatedly selfed Samsoun tobacco line; note necrotic lesions in all leaves of plants possessing the dominant gene N. E. Young nn Burley backcross plant showing systemic chlorosis. F. Similar young Nn plant showing systemic necrosis; contrast with E. G. Older nn plant showing systemic chlorosis. H. Comparable Nn plant showing only local necrosis at site of inoculation. E-G. Inoculated leaf at left.

temic necrosis proved troublesome. It was found, however, that, as plants became slightly older, there was an increasing delay of systemic infection in the presence of the gene for necrosis. It was then possible, after inoculating, to allow systemic chlorosis to appear in all nn plants before safeguarding necrotic-type plants by cutting away the inoculated leaves to prevent subsequent escape of virus into stems and top leaves. In still older plants inoculated leaves could be left attached indefinitely without escape of virus to other parts.

Observed deficiencies of necrotic-type plants in progenies derived from Connecticut Broadleaf tobacco, as shown in the third column of table 1, are not entirely understood. Successive generations have shown no indication of any tendency toward establishment of the gene N. No simple 1:1 backcross or 3:1 selfed ratios have occurred thus far. It seems probable, however, that a subsequent approach from a different angle, by hybridization of the necrotic-type Burley and Samsoun derivatives with Connecticut Broadleaf tobacco, may permit the necrotic-type gene to be incorporated in this third variety also.

In figure 1, leaves and plants of necrotic and chlorotic types are represented as they appeared at certain intervals after inoculation. The tendency to virus localization in necrotic-type plants is illustrated.

HOMOZYGOUS NECROTIC-TYPE LINES OF NICOTIANA TABACUM

After the first backcross generation there was little visible indication of any characteristic of *Nicotiana glutinosa* in the hybrids. In successive generations of crosses of necrotic-type plants with 3 varieties of *N. tabacum*, the distinctive characteristics of the 3 horticultural types were soon acquired.

For the purpose of obtaining plants homozygous with respect to the gene N, selfed lines were instituted with necrotic-type plants of the first backcross generation as origin. No homozygous sets of plants were attained in the Connecticut Broadleaf selfed line, as might be expected from the failure of this line to produce normal monohybrid ratios. The selfed line of Burley yielded satisfactory 3:1 ratios, but this also has not yet given any progenies lacking chlorotic-type individuals. In the selfed line of Samsoun tobacco, however, sets consisting only of necrotic-type plants were obtained. These obviously were derived from homozygous parent plants, in gametes of which the chromosome bearing the gene N had been included regularly enough to avoid formation of homozygous recessive-type plants in the tested sample of progeny. Whether complete gametic purity with respect to the gene N had been attained was an important question. A more sensitive test of this was given by reciprocal backcrosses to a chlorotic-type tobacco plant.

The apparently homozygous necrotic-type (NN) Samsoun tobacco derivative, which produced 339 necrotic-type : 0 chlorotic-type plants in the F₂ generation (Table 1), was crossed reciprocally with a plant of the original chlorotic-type variety Samsoun (nn), using an individual of the F₂ generation first as pollen parent, then as seed parent. Selfed progeny of the Sam-

soum plant were found to be consistently of chlorotic type (250 individuals tested). Selfed progeny of the F_3 plant were those reported in the table as the F_4 generation (303 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 here described, with desired types as recurrent parents, or better by similar crosses of necrotic-type, Nn plants of the B_4 generation described in this paper (see table 1). These B_4 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 imprisonment 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. Valleau (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 Ambalema 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 contaminative 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 Ambalema 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 protecting 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-

though its effect in preventing the mottling manifestations of classical tobacco-mosaic disease was the first to be noted, by Allard in 1914 (1, p. 14), this function was not the first to be exploited. Its first important contribution to the scientific investigation of tobacco-mosaic disease was in facilitating quantitative measurement of the causative virus by inducing prompt formation of conspicuous primary lesions, which in great number on a given leaf surface indicated high titer of virus, but, in less abundance, indicated lower titer. A similar gene of *N. rustica* L. and a mechanism of unknown genetic nature in *Phaseolus vulgaris* L. also have been utilized for the same purpose. The ease of performing quantitative measurements thus gained has allowed unusually extensive as well as intensive studies of this virus to be carried out in the decade since 1928, the year in which this method of measurement began to replace earlier, but less efficient and less productive, techniques of minimal inoculation of *N. tabacum* and *N. rustica* plants. The second contribution of this dominant gene was in allowing accurate separation of strains of tobacco-mosaic virus. A similar gene of *N. langsdorffii* Weinm. has been used also for this purpose, permitting many newly derived strains to be promptly separated when they arose from strains already in hand. Its third contribution, in conferring a desirable type of disease resistance, though envisaged first and long delayed in accomplishment, may prove no less important through its apparently decisive control of epiphytotic spread of all strains of tobacco-mosaic virus in *N. tabacum*, the present outstanding source of contaminative infection for susceptible hosts of this virus among crop plants.

SUMMARY

A dominant gene *N*, inducing a necrotic type of response to infection with tobacco-mosaic virus, was transferred from *Nicotiana glutinosa*, through the medium of the amphidiploid species *N. digluta*, into strains of the species *N. tabacum*. By repeated backcrosses of necrotic-type hybrids to *N. tabacum*, tobacco-like derivatives of necrotic type were produced. A homozygous line was then attained by repeated selfings. This homozygous line is self-fertile and fertile with other strains of tobacco. The introduced gene is regularly distributed to its gametes, and so to all individuals of its progeny, whether obtained by selfing or hybridization. It is anticipated that it may prove feasible to incorporate this gene in locally acceptable types of tobacco, to prevent spread of virus from plant to plant within the tobacco crop, and so to eliminate the reservoir of virus in tobacco and tobacco products, the usual sources of infection both for succeeding crops of tobacco and for other crops susceptible to tobacco-mosaic disease.

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EFFECT OF SODIUM CITRATE ON RELEASE OF CURLY-TOP
VIRUS FROM ALCOHOLIC PRECIPITATE OF
PLANT JUICE¹

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INTRODUCTION

Certain phases of investigations on the chemical nature of resistance to curly top involved steam distillation of plant material in the presence of a small amount of sulphuric acid. *Atriplex semibaccata* Brown, *Chenopodium murale* L., and *Lycopersicon esculentum* Mill. were thus treated. The large volume of distillate that contained the plant acids was concentrated in each case under reduced pressure 1/20 the original weight of material used and these concentrated fractions were adjusted to pH 7.0.

Tests were made to determine whether or not these concentrated extracts would inactivate the curly-top virus. One ml. of juice from diseased beet plants was added to 4 ml. of each extract. These mixtures were allowed to stand at room temperature for a definite period, usually overnight. The amount of virus present in the mixture was then determined by the method worked out by Bennett.²

¹ Contribution from the United States Department of Agriculture, Bureau of Plant Industry, Division of Sugar Plant Investigations, Riverside, California.

² Juice was expressed from the severely diseased leaves of 5 to 15 beet plants and centrifuged. To 1-ml. aliquots was added an equal volume of 95 per cent ethyl alcohol. The resulting precipitate was thrown down by centrifugation and the supernatant liquid, discarded. The precipitate was washed once with 50 per cent alcohol, dried, and suspended in water and centrifuged. The supernatant liquid was made acceptable food for the leaf hoppers by adding sufficient sucrose to make a 5 per cent solution. The percentage of leaf hoppers that transmitted the virus from such a liquid to seedling sugar beets indicated