AN AGE-INDUCED VARIATION IN SUSCEPTIBILITY TO VIRUS X IN *NICOTIANA TABACUM* L.

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Summary

Stage of maturity influences the susceptibility of *N. tabacum* to virus X. This phenotypic variation is evidenced by a discontinuity of X infection in the inoculated leaves and a lack of systemic invasion of plants inoculated at a late growth stage compared with a complete infection of the inoculated leaves and a rapid systemic invasion of plants inoculated at an early growth stage. Aging, even under conditions of very low nutrition, of plants inoculated at an early growth stage had no influence on their virus status. There was no indication that a virus X inhibitor was responsible for the reduced susceptibility to infection of old tobacco plants.

In view of the phenotypic variation induced by increasing maturity it is necessary to take into account this easily controllable factor when evaluating progenies in programmes covering the genetics of virus resistance.

I. INTRODUCTION

During the course of a genetical study of virus X resistance in the seedling progenies of a number of potato (*Solanum tuberosum* L.) crosses, the standard Brownell type X (Bald and White 1942) used for inoculation was cultured in either tobacco (*Nicotiana tabacum* L.) or *Datura stramonium* L. Although *D. stramonium* cultures were invariably satisfactory the results of the inoculum from the tobacco cultures were sometimes aberrant. This was evidenced by the failure of some of the control plants of the virus-free variety, Factor, to become infected. Normally young plants of this variety are completely susceptible to hand inoculation with virus X. It was concluded that virus X was either absent or in very low concentration in the tobacco plants responsible for the aberrant results.

Investigation of the problem revealed an interesting undescribed relationship between maturity of tobacco plants at time of inoculation with virus X and extent of systemic development of this virus. It is considered that the experiments on this relationship described in the present paper assist in the understanding of the phenotypic reactions that result in the resistance of plants to viruses. The phenotypic reaction of a plant to a virus is determined by the interaction between the genotype and the environment and one of the most important components of the environment in this connection is temperature. The effect of temperature on the reaction to the mosaic virus of tobacco hybrids developed

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from known genotypes has been described by McKinney and Clayton (1945). That non-environmental factors like plant vigour and maturity also influence the reaction to viruses of phenotypes derived from virus Y-resistant genotypes has been shown by Hutton (1948). Bawden and Roberts (1947), in their experiments on the influence of light on the susceptibility of tobacco, *Nicotiana glutinosa*, and tomato to several of a group of four viruses, noticed a gradient of increasing susceptibility from the oldest to the youngest leaves on the same plants and considered that in older plants and leaves this could be due to the accumulation of photosynthetic products inhibitory to virus multiplication. They did not work with virus X and used relatively young plants for all their experiments. Such relationships as have been described emphasize the need for a biochemical understanding of phenotypic reactions to viruses as a basis for the genetical study of virus resistance in plants and for the development of new genetical methods for solving these problems.

II. Materials and Methods

For all the experiments described virus X was cultured in *D. stramonium*. This plant was used also as the main indicator for the presence of virus X. In the final experiments with Turkish tobacco and in the tests for the presence of an inactivating substance in old tobacco plants where a quantitative measure of virus was required, the globe amaranth (*Gomphrena globosa* L.) as described by Wilkinson and Blodgett (1948) was used.

In the first two series of experiments Brownell type X was used but in the later ones a virulent strain of X was employed to obviate possible complications from avirulent strains normally present in the Brownell type. Inoculum was prepared by macerating the leaf tissue in a mortar with a pestle at the constant proportion of 1 : 20 by weight with water. Where the pattern of virus development was being followed in the inoculated leaves 1 : 40 inoculum was used. All inoculations were done with a ground-glass spatula after dusting the leaves with fine carborundum powder.

III. Age of Tobacco Plants at Inoculation and Degree of Systemic Invasion by Potato Virus X, Using Tobacco Variety Kentucky 41A

Preliminary experiments with the tobacco variety Kentucky 41A indicated clearly that young plants invariably became systemically infected after inoculation of tip leaves with virus X. On the other hand, plants at or close to the flowering stage rarely became invaded systemically when inoculated. Even the introduction of virus by grafting did not result in a full systemic invasion of old plants. This was demonstrated by an experiment in which two young tobacco plants four weeks from transplanting, and six old tobacco plants 14 weeks from transplanting, were grafted towards the base with Brownell potato scions. At monthly intervals over a period of four months portions of the tip leaf and of the basal leaf of the tobacco plants were inoculated separately to duplicate plants of *D. stramonium*. The young plants became systemically infected with virus

X within the first month. In the old plants there was no movement of Brownell X to the tips but a gradual invasion of all the basal leaves within three months.

A detailed experiment extending over 10 months was done to discover more about the principles involved in the apparent resistance of old tobacco plants to systemic invasion by virus X. Fifteen young tobacco plants were transplanted and divided equally into five groups. At 4, 8, 14, 19, and 26 weeks respectively after transplanting, one of these groups was inoculated once on the young tip leaves with Brownell X. When inoculated the approximate heights of the five groups were 10, 24, 35, 41, and 45 in. respectively, the third group being near flowering, and the last two groups at the flowering stage. At 10-weekly intervals after plants were inoculated, portions of the youngest tip leaf and of the basal leaf were each inoculated separately to duplicate plants of *D. stramonium.* Where the basal leaf had yellowed the next lowest green leaf was used. If the indicator plants remained free of symptoms they were reinoculated with a virulent strain of X to test for the presence of masked strains.

TABLE 1

EFFECT OF AGE OF TOBACCO PLANT AT INOCULATION ON SYSTEMIC INVASION BY VIRUS X AS SHOWN BY TRANSFERS TO *DATURA STRAMONIUM* L.

Group	Tobacco Plant Nos.	No. of Weeks After Transplanting when Inoculated	Results of Transfers to *D. stramonium** at 10-weekly Intervals after Inoculation of Tobacco Plants							
			1		2		3		4	
			Tip	Base	Tip	Base	Tip	Stem	Tip	Stem
1	1,2,3	4	+	+	+	+	+		+	+
2	4,5,6	8	4 + 5&6 O	+ O	4 + 5&6 O	+ O	+ +	+ +		
3	7,8,9	14	O	O	O	O	O	O		
4	10,11,12	19	O	O	O	Stem O				
5	13,14,15	26	O	O	O	Stem O				

* + Signifies presence of, and O absence of, virus X in *D. stramonium.*

In no case was a masked strain found. After the fourth transfer from their tip leaves to *D. stramonium,* the first group of tobacco plants inoculated were cut back to a height of 14 in. above the soil in the pot, a small piece of stem at this height then being inoculated to duplicate plants of the indicator. The same procedure was adopted for the second and third groups of tobacco plants inoculated after the third transfer to *D. stramonium,* and for the fourth and fifth groups after the second transfer. Before cutting back, the tobacco plants of the first and second groups were 24-30 in. in height while the rest of the groups varied from 48 to 60 in. above the pots. These differences were in themselves striking evidence of systemic invasion by virus X in the first two groups and its retarded development in the last three groups.

From the results in Table 1 it can be seen that systemic invasion by virus X occurred in the tobacco plants inoculated four weeks after transplanting, while one of those inoculated eight weeks after transplanting was readily invaded, and in the other two plants of this second group systemic development of the virus was retarded and did not occur until 20-30 weeks after inoculation. Evidently in these latter two plants physiological conditions were unfavourable to the development of virus X, and the fact that systemic invasion did not occur at all in the plants inoculated 14, 19, and 26 weeks after transplanting indicates that at eight weeks after transplanting the factors inhibiting systemic virus development had almost outbalanced those encouraging it. In Table 1 it is apparent that, once systemic invasion with virus X has occurred in young plants like those in group 1, their subsequent aging does not influence their virus status. In addition it is of interest to note that, following the inoculations from stem pieces obtained by cutting back the plants, the young leaf regrowth was inoculated to duplicate plants of *D. stramonium* with similar results to those obtained from the stem material.

IV. Effect of Nutrition on the Virus Status of Tobacco Plants Infected with X at an Early Growth Stage

As the previous experiments had shown that tobacco plants in their later growth stages were resistant to systemic invasion by virus X it was of interest to see whether it was possible to influence the virus status of old plants that had been infected at an early growth stage. Table 1 indicated that aging in itself did not influence the virus content of plants inoculated early in life so it was decided to use a combination of aging and low level of nutrition.

Ten young tobacco plants of the variety Kentucky 41A were inoculated with Brownell type X, and six weeks later when they were strongly mottled they were transplanted to washed river sand and given water only. At monthly intervals over a 12-months period, portions of the tip leaves of each plant were inoculated to duplicate *D. stramonium* plants. In spite of the severe effect of aging at a very low level of nutrition on growth and metabolism the indicator plants always showed the presence of virus X. None of the tobacco plants grew more than 12 in. high and the leaves were dwarfed and intensely chlorotic. Under these conditions the symptoms of virus X disappeared but reappeared for a short period after an application of potassium nitrate half way through the experiment.

It is apparent that, when virus X development becomes an integral part of the protein metabolism of a young tobacco plant, aging in rich potting soil as in the experiment of Table 1, or aging under conditions of very low nutrition do not effect a change in the phenotype. However, if a plant is aged before inoculation with virus X, a phenotypic variation is induced resulting in a physiological state inhibitory to virus development.

V. Pattern of Virus X Development in Old and Young Plants of Tobacco Variety Kentucky 41A

In order to gain some understanding of the age-induced phenotypic variation in virus X susceptibility in the tobacco variety Kentucky 41A, the pattern of virus development in the inoculated leaves of young and old plants was investigated. Three young, vigorously growing plants 9-10 in. high at four weeks after transplanting and two old plants 14 weeks from transplanting and at the flowering stage and 35-40 in. high were selected. Inoculum of a virulent strain of X was prepared by macerating 1 g. of leaf tissue in 40 ml. of water. Two leaves of each of the young plants and six leaves of equivalent size on each of the old plants were tagged and inoculated. Five days after inoculation a systemic mottle had developed in the young plants, but no systemic symptoms were present in the old plants. Twelve days after inoculation young tip leaves of each of the five tobacco plants were inoculated to duplicate *D. stramonium* plants.

Table 2

EXTENT OF DEVELOPMENT OF A VIRULENT STRAIN OF X IN LEAVES OF OLD AND YOUNG KENTUCKY 41A TOBACCO PLANTS AFTER THEIR INOCULATION, AS SHOWN BY A TISSUE SAMPLING METHOD

Tobacco Plants			Number of Tissue Discs Out of 12 Giving Virus X	
No.	Weeks from Transplanting	Inoculated Leaves Used	12 Days after Inoculation	30 Days after Inoculation
1	14	Top	6	11
		Lower	8	10
2	14	Top	9	11
		Lower	5	11
3	4	One	12	12
4	4	One	12	12
5	4	One	12	12

At the same time as the tip leaves were sampled, 12 discs of tissue 4 mm. in diameter were removed with a cork borer from each of seven inoculated leaves, four of these being on the two old plants and the rest of the leaves being distributed among the three young plants. The tobacco leaves were of similar size and ranged in area from 225 to 280 sq. cm. Plate 1 shows the 12 discs removed from one of the tobacco leaves and the manner in which they were distributed. Each disc of tissue was inoculated to a single *D. stramonium* plant.

A month after inoculation of the two old and three young tobacco plants a similar series of discs was removed from another set of their inoculated leaves. As before, portions of the young tip leaves were inoculated to duplicate *D. stramonium* plants at the same time as the discs of tissue were inoculated to single plants of this indicator. The results of the two series of inoculations are given in Table 2.

It will be seen in Table 2 that, 12 days after inoculation, none of the discs taken from the inoculated leaves of the young plants was free of X whereas 25-58 per cent. of those taken from the inoculated leaves of the old plants were free of X as measured by infection in *D. stramonium*. At this stage a definite systemic mottle was present in the young plants while no systemic movement of X was detected in the old plants. Thirty days after inoculation a similar position applied in the young plants but in the old plants a further development of virus X had taken place so that only 8-16 per cent. of the tissue discs from the inoculated leaves were free of X. Even this indicates considerable discontinuity of virus in the leaves of the old plants and fits into the general picture of an age-induced phenotypic variation in susceptibility to virus X. As before, no systemic movement of virus X to the young tip leaves had occurred in the old plants.

After these results were obtained, the stems of all the tobacco plants were cut into six equal lengths, each of which was inoculated to duplicate *D. stramonium* plants. The position with respect to presence or absence of X in these stem portions is shown in Table 3.

TABLE 3

VIRUS X STATUS OF THE STEMS OF OLD AND YOUNG KENTUCKY 41*A* TOBACCO PLANTS AT SUCCESSIVE SITES ALONG THEIR LENGTH FOLLOWING LEAF INOCULATION

Tobacco Plants							
	Weeks from Trans-	Base		Mid Stem		Tip	
No.	planting	1	2	3	4	5	6
1	14	O	+	+	+	O	O
2	14	O	O	O	O	O	O
3, 4, 5	4	+	+	+	+	+	+

+ Signifies presence of, and O absence of, virus X in *D. stramonium*.

From the evidence in Table 3 it is apparent that inhibition of virus X multiplication occurred in the stems of the two old tobacco plants inoculated on the leaves with virus X 14 weeks after transplanting. In plant 1 the virus had moved from the inoculated leaves into the corresponding stem areas, resulting in a limited development in these areas without migration to the rest of the stem. In plant 2 movement of virus X from the inoculated leaves may not have occurred, and if it did, there was a total inhibition of its development in the stem. As would be expected from the previous results, a free development of virus X occurred in the stems of the young plants 3, 4, and 5. It is thus clear from the results of Tables 2 and 3 that inhibition of virus X multiplication is common to both stem and leaf tissue of tobacco plants inoculated 14 weeks from transplanting.

VI. Virus X Development in a Turkish Variety of Tobacco

As all the previous experiments were done with the tobacco variety Kentucky 41*A* it was necessary to compare the results of virus X inoculations in another variety with an inherently different phenotype. The Turkish type of tobacco, with its large number of short leaves and compact growth habit, was chosen as being inherently different from Kentucky 41*A*, with its fewer larger leaves and spreading growth.

Six flowering Turkish tobacco plants averaging 56 in. high, which had been transplanted 14 weeks earlier were each inoculated on six leaves with virulent X. With plants 1, 2, and 3 the inoculated leaves were along the lower half of the stem and in plants 4, 5, and 6 they were along the upper half of the stem. Three young Turkish tobacco plants four weeks from transplanting, and averaging 12 in. high, were each inoculated on three leaves as controls. A month later three or four discs of tissue 4 mm. in diameter were removed from a proportion of the inoculated leaves with a cork borer. All the inoculated leaves could not be sampled because the reaction to virus X of a number of them had resulted in the death and yellowing of a considerable area of the tissue. Only leaves of a normal green colour were sampled. Each disc of leaf tissue was macerated in two drops of water and inoculated to two small *D. stramonium* plants and half a globe amaranth leaf. After this sampling was completed, portions of the lowest uninoculated leaf and of the tip leaf were each inoculated to duplicate *D. stramonium* plants. The results are given in Table 4.

Table 4

RESULTS FROM THE VIRUS X INOCULATIONS OF OLD (NOS. 1-6) AND YOUNG (NOS. 7-9) TURKISH TOBACCO PLANTS AS SHOWN BY TISSUE SAMPLING AND TRANSFER TO *D. STRAMONIUM* AND GLOBE AMARANTH

Plant No.	No. of Leaves Sampled	No. of Tissue Discs Taken	No. of Tissue Discs Without X Indicated by *D. stramonium*	Mean No. Lesions per Half Leaf of Globe Amaranth	Systemic Movement of X (indicated by *D. stramonium*) to:	
					Basal Leaf	Tip Leaf
1	6	24	0	204	0	0
2	3	12	0	172	0	0
3	2	6	0	112	+	0
4	6	18	0	118	0	0
5	5	15	4	87	0	0
6	6	18	1	123	0	0
7,8,9	3	12	0	156	+	+

The results in Table 4 with Turkish tobacco agree with those described earlier in this paper for Kentucky 41*A*. It is apparent that systemic movement of virus X from the inoculated leaves of the old plants was rare and that virus development was inhibited in the leaves of plants 5 and 6 as evidenced by the

absence of virus X in 27 per cent. and 6 per cent. of the tissue discs respectively. The mean numbers of lesions per half leaf of globe amaranth have little significance but there may be a correlation with plant 5, which gave the greatest number of leaf discs free of X and the lowest mean number of globe amaranth lesions.

After the results of Table 4 were obtained, the old Turkish tobacco plants which then averaged 65 in. high and the young plants 24 in. high were stripped of their leaves and the stems cut into six equal pieces, each of which was inoculated to duplicate *D. stramonium* plants. The results are given in Table 5.

TABLE 5

VIRUS X STATUS OF THE STEMS OF OLD AND YOUNG TURKISH TOBACCO PLANTS AT SUCCESSIVE SITES ALONG THEIR LENGTH FOLLOWING LEAF INOCULATION

Plant No.	Base		Mid Stem		Tip	
	1	2	3	4	5	6
1	O	+	O	O	O	O
2	O	O	O	O	O	O
3	O	+	O	O	O	O
4	O	O	O	+	+	O
5	O	O	O	O	O	O
6	O	O	O	+	+	+
7,8,9	+	+	+	+	+	+

+ Signifies presence of, and O absence of, virus X in *D. stramonium*.

In Table 5 much the same situation is shown as applied previously with Kentucky 41A. Movement of virus X into the stem tissue of the old plants 1-6 was infrequent, but where it occurred it was associated with the position of the inoculated leaves. In Table 5, plants 1, 2, and 3 were inoculated on basal leaves and plants 4, 5, and 6 on leaves in the upper half of the plant. There is some indication of a greater movement of virus X into stem tissue when leaves on the upper half of the plant are inoculated.

Tables 4 and 5, in conjunction with the previous tables, suggest strongly that this age-induced phenotypic variation in virus X suspectibility is common to *Nicotiana tabacum* varieties irrespective of the genotype. Although genetical experiments have not been made it can be assumed from definite differences in growth habit that the genotypes of Kentucky 41A and Turkish tobacco varieties are also quite different.

VII. Tests for the Presence of a Virus X Inhibitor in the Juice from Leaves of Old Tobacco Plants

There seemed to be two possible explanations for the phenotypic variation in virus X susceptibility induced by age in tobacco plants. One supposed that the rate of production of inhibitory substances increases with maturity and the

other that the ontogenetic drift in the protein metabolism of aging plants results in changes that restrict the ribose nucleic acid synthesis associated with virus X multiplication. Bawden and Roberts (1947) inoculated plants 5-6 weeks after transplanting with viruses that did not include X and observed that on the same plant young leaves were more susceptible than old, and considered that the differences could be due to a greater accumulation of photosynthetic products in older leaf tissue.

In order to investigate the possibility that the reduced susceptibility of aged plants to virus X was due to a relatively high percentage of inactivating substances, as opposed to a low percentage in young plants, an experiment was planned using the tobacco variety Kentucky 41A, a virulent strain of X in *D. stramonium*, and the quantitative indicator globe amaranth. Two 1 g. lots of *D. stramonium* leaf containing the virulent X strain were taken from the same plant, and one was macerated with 1 g. of leaf from a tobacco plant 14 weeks from transplanting and the other with 1 g. of leaf from a plant that had been transplanted four weeks previously, both lots being made up to 40 ml. with water. Each inoculum was wiped on separate sets of six globe amaranth leaves immediately after mixing, and then again to further sets of six leaves of this indicator after standing for 4, 8, and 16 hours. At each of these three time intervals controls were freshly made from the leaves of old and young tobacco plants as before and inoculated immediately to sets of six globe amaranth leaves. Owing to a lack of sufficient globe amaranth plants, proper randomization of the inoculated leaves could not be obtained, but sufficient comparisons and replications were made to show trends. The results are given in Table 6.

TABLE 6

MEAN NUMBER OF LESIONS ON GLOBE AMARANTH LEAVES FROM INOCULA MADE BY MACERATING X-INFECTED *D. STRAMONIUM* WITH OLD AND YOUNG TOBACCO LEAF RESPECTIVELY

D. stramonium Containing X Mixed with	Mean No. Lesions on Globe Amaranth Leaves from Inoculum			
	Immed. After Mixing	After 4 hr.	After 8 hr.	After 16 hr.
Old tobacco leaf	83	91	62	51
Young tobacco leaf	55	52	43	40

Table 6 gives no indication of an excess of virus X-inactivating substance in old tobacco leaf since the amount of active virus in the inoculum containing it, as judged by the number of lesions on globe amaranth, is greater than in the inoculum made with young leaf. The trend is more in the direction of greater quantities of inactivating substance occurring in young leaf although the steady inactivation with time is more apparent in the inoculum containing old leaf tissue. Further and more detailed experiments would be needed to

discover whether these are real differences or not. The three pairs of controls for the 4, 8, and 16 hour treatments in Table 6 confirmed the finding that, immediately after mixing, the inoculum containing old tobacco leaf gave a greater number of lesions on globe amaranth than that containing young leaf, the mean difference being 20 lesions.

These results suggest that the cause for the reduced susceptibility of tobacco plants to virus X with age is resident in a changed metabolism inhibitory to virus multiplication. It is unlikely that this relationship is dependent on the increasing production of inactivating substances in aging plants.

VIII. Discussion

The biochemical reactions of plants to viruses are complex and require considerable research before they will be understood. However, much can be done by studying the effects of the major controllable factors on plant reaction to viruses. Environmental factors like temperature and light have been studied in this connection by various workers and this paper describes the effect of the non-environmental factor maturity on the interaction between virus X and *N. tabacum*. Such knowledge is an essential basis for research programmes involving studies of the genetics of virus resistance in plants. The testing of progenies in these programmes is often done under relatively uncontrolled conditions. If the factors needing control are known and attention is given to them, masking of the influence of the genotype on the phenotypic reaction to virus is reduced considerably so that genetic ratios can be determined with greater accuracy. The influence of maturity on phenotypic reactions to viruses is not generally realized, and experience has shown the importance of this easily controlled factor in the evaluation of progenies for virus resistance. In some virus-plant combinations, as with virus Y in the potato, the interaction works in the opposite direction to that described in this paper so that more mature plants are needed to accurately assess the inherent resistance of hybrid progeny.

Perhaps the most interesting feature of the results described in this paper is the marked difference in virus status between old plants inoculated at early and late growth stages respectively. Apparently the introduction of virus X into young plants so alters their metabolism that the changes induced in uninoculated plants by aging are inoperative. It may be that the ontogenetic drift in the protein metabolism of tobacco plants inoculated when young is so changed, that the plants are in a continually susceptible condition when compared with old uninoculated plants. If growth is any indication of the rate of virus production in the plants inoculated at an early growth stage, it could be assumed that the rate slows down considerably with age. The points raised pose some interesting biochemical problems, the solution of which could lead to an understanding of what makes plants resistant to virus infection.

IX. Acknowledgments

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X. References

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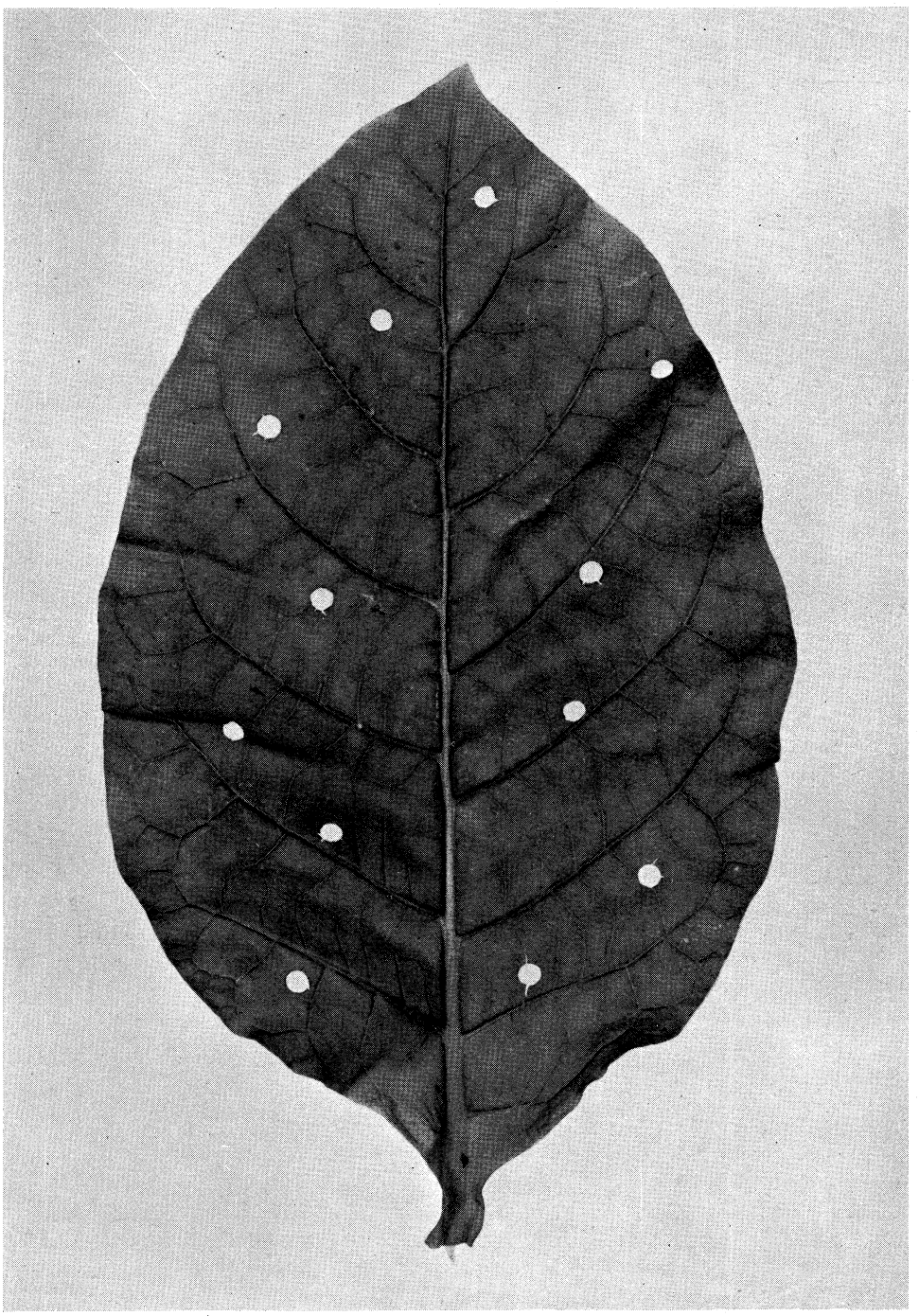

An X-infected tobacco leaf with 12 tissue discs, each 4 mm. in diameter, removed for determining the pattern