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THE EFFECT OF „PREMUNITY" ON THE TYPE OF LESIONS INDUCED BY AUCUBA MOSAIC VIRUS

Met een samenvatting: Het effect van premunisatie op het type vlekken te weeg gebracht door het aucubamozaiekvirus

BY

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INTRODUCTION

Since THUNG (8) directed attention to the antagonistic action of „green" and „white" strains of tobacco mosaic virus within the same plant, the phenomenon of „cross-immunity" has been studied under various angles and names. Following the suggestion of QUANJER (6), we shall use, in the present paper, the terms „premunity" and to „premunize" for the proper denomination of this phenomenon. Concerning the „premunizing" of plants by tobacco mosaic virus against aucuba mosaic, we wish to point out firstly, that THUNG in his first publication has already stressed the fact that the protective effect of green mosaic virus strains upon the superinoculation with a white strain is not always complete but often only partial. Many authors have tried to determine the extent of the protective effect of the „premunizing" strain and have generally chosen the quantitative approach for solving this problem. Such quantitative studies, the technique of which has been developed by KUNKEL (5), were generally based upon a comparison of the number of necrotic spots induced by aucuba mosaic virus in leaves of „premunized" and „check" plants of *Nicotiana silvestris*. However, after superinoculation with a „white" strain a plant harboring mosaic virus of a „green" strain, not only exhibits a smaller number of spots, caused by this second inoculation, but the very type of the symptoms caused by the virus of the superinoculation suffers certain changes.

So far, it appears from the literature that this special type of symptom expression in „premunized" plants has not been given much attention.

SADASIVAN (7) states (p. 360), that „the aucuba mosaic lesions of plants infected with tobacco mosaic virus, in addition to being fewer, were smaller and had much more uneven edges". KOEHLER und HAUSCHILD (4, p. 99) quote a paper, then unpublished, by BODE, in which a description is given of the type of symptoms produced by E.N. virus on leaves of tobaccoplants „premunized" with the tobacco mosaic virus. According to KOEHLER and HAUSCHILD the

necrotic lesions caused by E.N. virus in healthy tobacco plants are rather big, whereas in leaves containing tobacco mosaic virus they are smaller und stop enlarging rather early.

Both these papers refer only to necrotic symptoms and do not define the conditions under which such a change of symptom expression was obtained.

It is the purpose of the present paper to furnish a few additional remarks on this problem.

MATERIALS AND METHODS

For these tests we used young plants of *Solanum Seaforthianum*, grown from seeds obtained on Oct. 11th. 1945 through the kindness of Prof. G. STAHEL, Director of the „Landbouwproefstation" Paramaribo, Suriname. The type of reaction of this species to infection with mosaic virus and aucuba mosaic virus recommended the use of this plant for experiments on „premunity". In fact we had verified in preliminary tests that in this plant the symptoms produced by „aucuba mosaic" (Marmor tabaci var. aucuba HOLMES, 1) differ considerably from those caused by the „common tobacco mosaic" (Marmor tabaci HOLMES). This latter virus does not induce any local symptoms in the inoculated leaves, producing only a very faint vein banding and a twisting of the leaflets in the young leaves. On the other hand, the aucuba mosaic virus causes discrete spots on the inoculated leaves, which may be circular and chlorotic or of the ring spot type with necrotic edges. The secondary symptoms consist also of white discrete rings, which are sometimes coalescent, forming fern leaf pattern. Fig. 1 illustrates

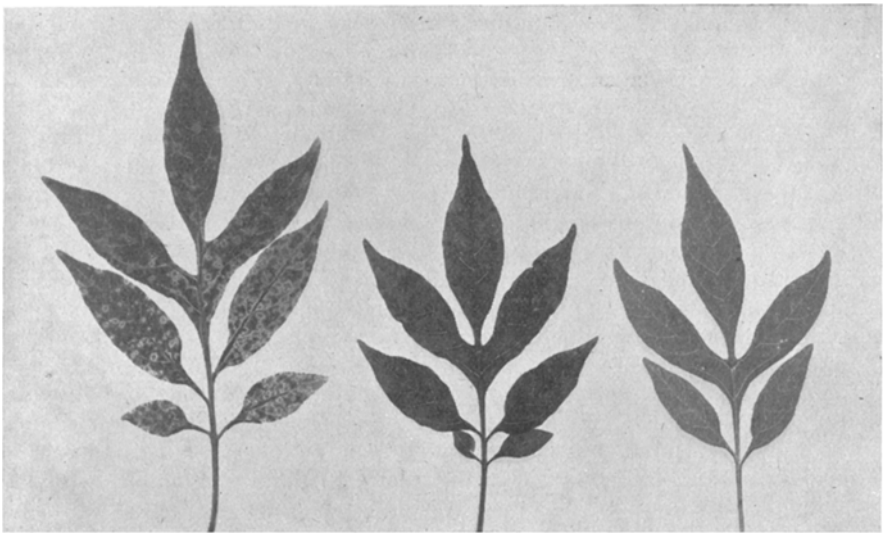

Fig. 1. Leaf of a healthy „check" plant of Solanum Seaforthianum (right) and leaves of plants of the same species systemically infected by tobacco mosaic virus (center) and aucuba mosaic virus (left). Inoculation performed on March 18, 1946; Phot. April 24.

Blad van een gezonde contrôleplant van Solanum Seaforthianum (rechts) en bladeren van planten van dezelfde soort, die systemisch geïnfecteerd zijn met tabaksmozaiekvirus (midden) en aucubamozaiekvirus (links).

the secondary symptoms induced in leaves of *Solanum Seaforthianum* by aucuba mosaic (left) and tobacco mosaic (center). On the right, is shown a leaf of a healthy „check" plant, for comparison.

As we intended to study the „local" and the „systemic" effect of „premunity", our general procedure consisted in mechanically inoculating one leaf of each young plant – for instance the 4th leaf counted from the apex – with sap containing tobacco mosaic virus and in performing the superinoculation with sap containing aucuba mosaic virus about 3 days later on the same leaf as well as on the next younger one (which had not been rubbed with the tobacco mosaic-containing sap). At the same time the identical number of plants, which three days earlier had been treated with distilled water and not with tobacco mosaic virus-containing sap, received an inoculation with aucuba mosaic virus.

Sometimes, as will be explained in the section describing our results, this general procedure was modified for special experimental purposes.

EXPERIMENTAL

Before we attempt to define the conditions which are favorable for the appearance of the „modified" symptoms of aucuba mosaic, we shall describe in some detail the first series of experiments, the results of which drew our attention to these symptoms.

The first experiment was performed on Jan. 29, 1946. On this date 40 young plants of *Solanum Seaforthianum* were set apart for inoculation tests. Twelve of the plants were rubbed on two successive leaves with sap of healthy plants of *S. Seaforthianum*, while 12 other plants were inoculated on corresponding leaves with sap of the same species containing tobacco mosaic virus and, finally, 12 other ones received an inoculation with aucuba mosaic-containing sap of *S. Seaforthianum*. On Feb. 1, the superinoculation was performed, always on the upper of the two leaves which had been treated with sap previously and on one young leaf which had not been touched during the earlier treatment. Four plants (of each group of 12) were not superinoculated, to serve as checks, 4 others were rubbed with sap containing tobacco mosaic virus and the remaining 4 were inoculated with sap containing aucuba mosaic virus. Finally, a group of 4 plants, which had not been inoculated previously, was inoculated with the mixture of the saps containing the two viruses. The 4 groups of plants which had not received any inoculation with aucuba mosaic virus-containing sap, remained entirely free from symptoms of the annular type. In the group of plants inoculated only with aucuba mosaic the 3 successive leaves displayed a great number of annular spots, viz. 284, 310, 232 respectively. The number of these spots was smaller in the group of plants which had been treated first with sap of healthy *S. Seaforthianum* and had then received the inoculation with aucuba mosaic virus. The breaking of the hairs previous to the inoculation may in this case be responsible for the reduction of the number of lesions. The numbers of lesions (on the basal, medium and apical leaves) were in this group 0, 90 and 146 spots respectively. Still smaller was the number of annular spots in the group of plants „premunized" first with tobacco mosaic virus and afterwards superinoculated with aucuba. The numbers of spots counted in this group were 0, 68 and 93, respectively. In order to complete this picture it should be mentioned that in the group of plants „premunized" with aucuba mosaic virus and superinoculated with tobacco

mosaic virus only the upper leaf displayed a certain reduction in the number of annular spots and that in the group of plants inoculated with the mixture of the two viruses the symptoms of tobacco mosaic were predominating (SADASIVAN 7, 1940, pag. 361). We are only concerned here with the comparison of the type of the symptoms occurring in the plants of the various groups. On February 14 we observed that the leaves of plants first „premunized" with tobacco mosaic virus and then superinoculated with aucuba virus preserved for a long time their normal green colour. The spots exhibited by these leaves had clear-cut border lines, which marked the limit between the yellow spots and the green leaf area very precisely. In the „check" leaves, which did not display any „premunity". these spots, frequently consisting of concentric rings, expanded over a prolonged period, slowly turning the whole leaf surface chlorotic.

This difference in the behaviour of the spots between normal and „premunized" leaves was also shown in our experiment of March 18, 1946. In this case a procedure was followed which differed from that of the former experiment. Twenty four plants of *Solanum Seaforthianum*, were divided into two groups of 12 plants each. On March 18 one leaf of each plant of the first group was rubbed with the sap of healthy tobacco leaves, whereas a corresponding leaf of each plant of the second group was inoculated with tobacco mosaic virus. The plants of both groups were then divided into 3 subgroups. Four plants of each of the original groups received the superinoculation with aucuba mosaic virus one day after the first treatment; a second batch of 4 plants of each group was superinoculated one day later, while in the remaining group, 3 days elapsed between the first treatment and the superinoculation. In this experiment we restricted ourselves to the observation of the local effect of „premunity", performing the „previous" and the „succeeding" inoculation on the same leaf.

The special purpose of this experiment consisted in determining whether or not the „premunity" has any influence upon the size of the annular spots which appear on the inoculated leaf. Therefore, on April 8, we counted the total number of spots as well as the number of „large" spots, i.e. those measuring at least 2 mm in diameter. The results of this experiment are represented in table 1.

TABLE 1

Total number of spots and number of „large" spots counted on (a) chek leaves, and (b) „premunized" leaves of Solanum Seaforthianum after inoculation with aucuba mosaic virus, performed on different days

group of leaves	Intervals between the two inoculations (days)	total number of spots	number of „large" spots
a = check	1	175	175
a	2	107	107
a	3	223	112
b = „premunized"	1	119	39
b	2	79	10
b	3	136	0

It is shown by the figures of this table that, in „premunized" leaves, the decrease in the number of „large" spots is much more pronounced than the drop in the general number of spots. With increase of the time interval between

the two inoculations, the protective effect of the first virus becomes more and more noticeable, as has been shown with another type of spot by different authors, for instance KUNKEL (5, p. 452) and SADASIVAN (7, p. 360). The difference between their case and ours lies in the fact that the spots produced by aucuba mosaic on leaves of *Solanum Seaforthianum* are essentially chlorotic, whereas the same virus on leaves of *Nicotiana silvestris* induces necrotic („dead") spots. This difference of the type of symptom probably reflects a major difference in the behaviour of the virus in both species. In leaves of *N. silvestris* the aucuba mosaic virus kills the cells, which become invaded by it. In *S. Seaforthianum*, on the other hand, the majority of the infected cells remain alive, allowing further multiplication of the virus. Perhaps that is the reason why, on leaves of *S. Seaforthianum* inoculated by aucuba mosaic, in spite of the „premunity", a certain number of small spots were observed. But the increase in diameter of these spots was inhibited, and this inhibiting effect was the stronger the greater the interval between the two inoculations.

The difference in the character of the spots exhibited by „check" and by „premunized" leaves of *S. Seaforthianum* is illustrated by fig. 2. The big leaf at the right is a leaf of a „check" plant, showing several „large" spots specially on

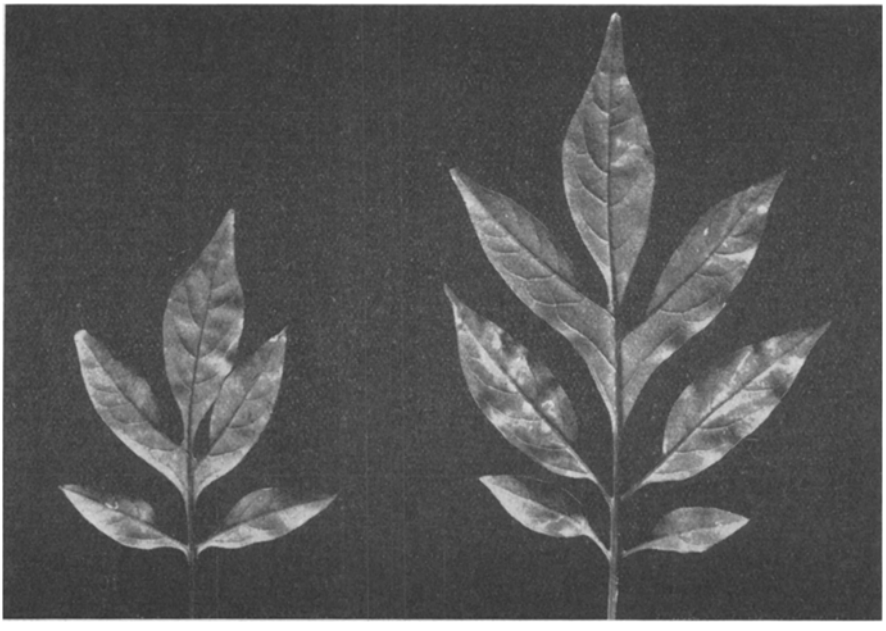

Fig. 2. Leaf of a „premunized" (left) and a „check" plant (right), both of them superinoculated on March 21 with the virus of aucuba mosaic. The „premunized" leaf shows a few small spots, the „check" plant (principally on one leaflet) large concentric ring-spots. Exper. of March 18, 1946; Phot. April 11.
Blad van een gepremuniseerde plant (links) en van een contrôleplant (rechts), beide op 21 Maart extra geinoculeerd met het virus van het aucubamozaiek. Het gepremuniseerde blad vertoont weinig kleine vlekken, de contrôle (hoofdzakelijk op een blaadje) grote concentrische ringen.

the second leaflet from the base. The small leaf at the left has been „premunized" and is showing various small spots.

The later development of the spots of „check" leaves and „premunized" leaves of the same series can be seen in our figs 3 and 4, of one month later. In the „check" leaves the spots have greatly extended, and the chlorosis covers practically the whole leaf. In the „premunized" leaves the spots did not increase so much. They are more intensely chlorotic but are separated by a well defined border from the rest of the leaf-area, which remains green.

This difference in character of the leaf spots between „check" and „premunized" plants was also confirmed by the results of an experiment which was begun on April 23, 1948. Then two groups were formed, each consisting of twelve young plants of *Solanum Seaforthianum*. One leaf of each plant of the first group was rubbed with distilled water and the corresponding leaf of each plant of the second group with sap of mosaic-infected tobacco plants. Three days later half of the plants of each group was „superinoculated" with sap containing aucuba mosaic virus. This superinoculation was performed on the leaf rubbed previously, and on a younger leaf which had not been touched before. From the spots which appeared on the older leaf we could draw conclusions as to effect of „local

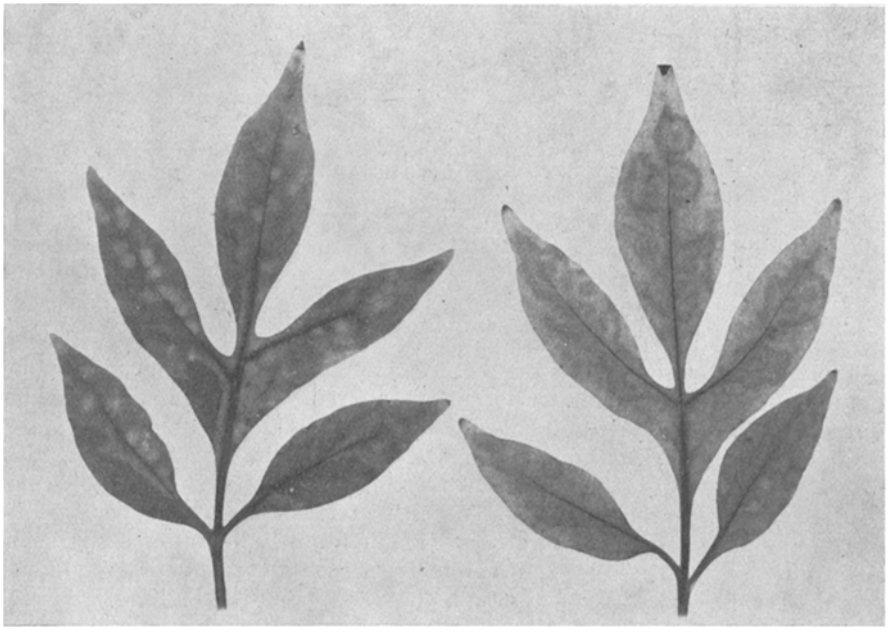

Fig. 3. Leaves of two „check" plants, 8 weeks after inoculation with the virus of aucuba mosaic. The spots have greatly enlarged, covering (especially on the leaf at the right) a considerable part of the blade, which displays a chlorotic colour. Exper. of March 18, 1946; Phot. May 14.

Bladeren van twee contrôleplanten, 8 weken na de inoculatie met aucubamozaiekvirus. De vlekken hebben zich sterk uitgebreid en bedekken vooral op het blad rechts een aanzienlijk deel van de bladschijf, die er chlorotisch uitziet.

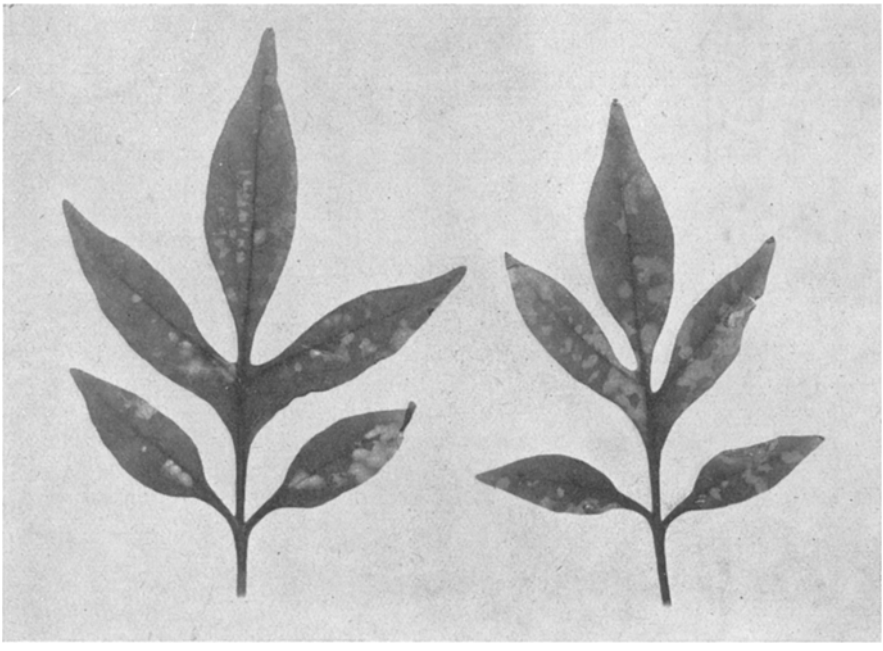

Fig. 4. Leaves of two „premunized" plants, comparable with those shown in fig. 3. The spots are small, intensively coloured, sometimes of irregular shape and stand out against the dark green of the unaffected leaf area. Exper. of March 18, 1946; Phot. May 14.

Bladeren van twee gepremuniseerde planten, die overigens op dezelfde wijze behandeld zijn als die van fig. 3. De vlekken zijn klein, intensief gekleurd, soms onregelmatig van vorm en zij steken scherp af tegen het groen van het niet aangetaste bladoppervlak.

premunity", whereas the observations on the younger leaf permitted an estimation of the effect of „systemic premunity".

Fig. 5, taken on May 19, i.e. one month after the first inoculation, gives an idea of the type and distribution of the spots on the „younger leaf". The upper row shows leaves taken from „premunized" plants, and the lower row those of the corresponding „checks". The difference between both groups of leaves is especially remarkable when we compare the terminal leaflets. In the „check" leaves almost the whole leaf surface is „chlorotic", since the transition from the color of the spots to the color of the remaining part of the leaf blade is gradual. In the „premunized" leaves – of which the third leaf from the left is the most typical one – the yellow leaf spots stand out against the dark-green color of the nonaffected area of the leaf blade.

It seems that the effect of „premunity" on the type of symptoms produced by aucuba mosaic virus is practically the same in locally and in systemically „premunized" leaves. While figures 3 and 4 show the result of an experiment on local „premunity", the leaves shown in fig. 5 hat not been rubbed with sap previous to the inoculation with aucuba mosaic virus. This fig. demonstrates, then, the reaction of leaves displaying a „systemic premunity" acquired by

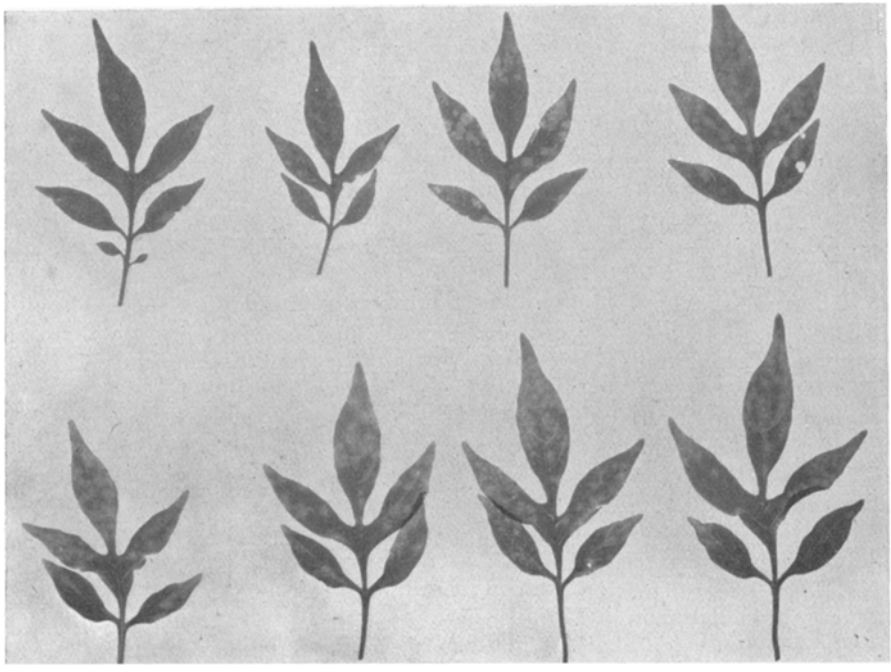

Fig. 5. Leaves of 4 „check" (lower row) and of 4 „premunized" plants (upper row), three weeks after their inoculation with aucuba mosaic virus. The leaves of the upper row had been „systemically premunized". The spots on the „check" leaves have increased more than those on the leaves of „premunized" plants. On the latter the spots are outstanding owing to the intensity of colour and the clear-cut borderline. Exper. of April 23, 1948; Phot. May 19.

Bladeren van 4 contrôleplanten (onder) en van 4 gepremuniseerde planten (boven) drie weken na inoculatie met aucubamozaiekvirus. De bladeren van de bovenste rij waren systemisch gepremuniseerd. De vlekken op de contrôlebladeren hebben zich meer uitgebreid dan die op de bladeren van de gepremuniseerde planten. Op deze laatste zijn de vlekken zeer opvallend als gevolg van de intensieve kleur en de scherpe rand.

infection with tobacco mosaic against a local superinoculation with aucuba mosaic virus.

In further experiments we attemped to study the type of symptoms occurring in systemically „premunized" leaves of *Solanum Seaforthianum* with still another experimental procedure. We shall mention here only the experiment performed on February 4, 1948 as an example of this series.

The essential difference between this test and the preceding experiments consisted in the fact that this time the leaf actually inoculated with aucuba mosaic virus was the only one which had remained without previous treatment. In other words, at the time of the first inoculation all the leaves of a plant, with one exception only, were rubbed either with distilled water (in the „check" group) or with tobacco mosaic virus-containing sap. In both groups the superinoculation performed three days later, was restricted to the leaf which had not suffered any treatment. The plants used in the experiment of February 4 consisted

of 21 individuals, 7 of which served as „checks", receiving a treatment with distilled water in all but one leaf. The other 14 plants were at the same time, and on corresponding leaves, „premunized" with sap containing tobacco mosaic virus.

The results obtained in this experiment, although in some points comparable with our previous observations, were rather surprising. The leaves of the „check" plants became chlorotic earlier than those of the „premunized" plants, and the spots appearing on them showed less contrast with the unaffected parts of the leaf blade than they did on the „premunized" plants. This had been expected.

On the other hand we had thought that the leaves of the „check" plants would exhibit the greater number of spots, especially many large annular spots. Actually, few such spots were formed on the leaves of the „check" plants and the total number of discrete spots was smaller than on the leaves of „premunized" plants. This result seems to be contradictory to our other observations. However, for a better understanding of the special behavior of the virus in leaves of „premunized" plants we may have to take into consideration the fact, that all the other leaves had been previously inoculated with tobacco mosaic virus and that, consequently, the aucuba mosaic virus may have remained restricted to the only leaf which, while it may have been to some extent systemically „premunized", was certainly not locally „premunized".

The effect of this „systemic premunity" was in our case probably not pronounced because the experimental leaf was situated between two groups of leaves already locally inoculated with tobacco mosaic virus. That would explain how a few foci of multiplication of the aucuba mosaic virus were able to develop on the „premunized" plants, but remained restricted to one leaf. In the „check" plants, on the other hand, the virus may have multiplied first in the inoculated leaf and may then have been translocated to the younger leaves. We found a certain support for this explanation in the occurrence of numerous white spots on young leaves of the „check" plants and in the lack, or the great scarcity, of such spots on the leaves of „premunized" plants.

Fig. 6 shows samples of leaves locally inoculated with aucuba mosaic virus. The two leaves on the right (chlorotic blades displaying few large-sized spots) were taken from „check" plants. The leaves on the left (dark-green blades displaying many small outstanding spots) belong to „premunized" plants. The difference in the type of symptom of aucuba mosaic in „check" and „premunized" plants respectively is again striking.

DISCUSSION

One of the most common symptoms induced by the virus of aucuba mosaic in leaves of *Solanum Seaforthianum* is a lesion of the type of „concentric ringspot". These lesions are characterized by alternating circular zones of necrotic and light green tissue. In the most typical cases there is found a central necrotic spot surrounded by a zone of living green tissue, which shows on its borderline a small necrotic ring which may be followed by other successive green and necrotic zones. The form of these lesions and their similarity with the so-called „Liesegang" phenomenon suggest, as many authors have pointed out, e.g. JOHNSON and VALLEAU (2), centrifugal spread of virus from small initial foci, comparable to the spread of solutions by diffusion in gels. KOEHLER (3) has studied

Fig. 6. Leaves of „check" (right) and „premunized" plants (left), 4 weeks after their inoculation with aucuba mosaic virus. The leaf of the „premunized" plants which was chosen for the superinoculation is in this case the only one which had not been previously rubbed with tobacco mosaic-containing sap. Large areas of the „check" leaves have turned chlorotic, whereas the „premunized" leaves have retained their dark green colour and present a rather high number of small „outstanding" spots. Exper. of February 4, 1948; Phot. March 9. (All pictures kindly taken by Mr Bruno Mazza, Photograph. Department of the „Instituto Biologico", SãoPaulo).

Bladeren van controleplanten (rechts) en gepremuniseerde planten (links) 4 weken na inoculatie met aucubamozaiekvirus. Het blad van de gepremuniseerde planten, dat gekozen werd voor de extra inoculatie, is in dit geval het enige blad, dat niet vooraf was ingewreven met sap van tabaksmozaiek. Grote plekken van de contrôlebladeren zien er chlorotisch uit, terwijl de gepremuniseerde bladeren hun donkergroene kleur hebben behouden en een vrij groot aantal kleine scherp afstekende vlekken vertonen.

the formation of ring spot symptoms on tobacco leaves more exhaustively, especially emphasising that the spread of the virus within these rings does not consist in diffusion only, since the virus continuously multiplies, principally along the outer border line of the lesions. In this respect there is a great difference between these lesions and the necrotic spots induced by some viruses on leaves

of „hypersensitive" species, since in this case the virus-multipication is more limited and restricted.

KOEHLER states as a working hypothesis for the explanation of the „ring-spot" symptoms, that the cells situated in the periphery of the rings are in a state of increased „sensitivity" and that they can be killed by some factor (possibly thermic) just at that time. With the further spread of the virus to a new „periphery", the cells lose again this sensitivity. In any case we may suppose that the multiplication of virus takes place chiefly in the green zone outside the most peripheral necrotic border line. In „check" leaves this circular spread of virus from initial foci may continue until nearly every cell belongs at one time or another, to the periphery of a „ring spot" lesion. Then the difference between affected and virus-free areas of the leaf blade disappears and the whole leaf acquires a slightly chlorotic aspect.

Let us consider for a moment what is likely to happen in „premunized" leaves: Since, in our experiments the interval between the „premunizing" inoculation and the „superinoculation" never exceeded a period of 3 days, and since we know from the papers of KUNKEL (5), SADASIVAN (7) and others, that the protective effect of the first virus becomes complete only after about 6 days, we are here chiefly concerned with „partially premunized" leaves. In such leaves the virus of the first infection is probably not yet distributed equally over the leaf surface. There may exist, however, several points, irregularly scattered over the leaf area, where virus particles became fixed to cells during the process of inoculation and these isolated cell groups may in the meantime have become centers of a circular virus multiplication. If we rub such leaves with sap containing aucuba mosaic virus (three days after those leaves had been inoculated with tobacco mosaic virus) many particles of the second virus may penetrate cells not yet invaded by the „premunizing" infectious agent. Part of these cells actually become infected with aucuba mosaic virus. These cells then become small centers of multiplication of this virus, which spreads as we have pointed out before in the form of alternating chlorotic and necrotic circular zones. Externally, these initial centers of aucuba mosaic virus multiplication can be recognized as small whitish points. In the course of the zonal increase of these centers it must so happen that the periphery of the circle reaches areas, already invaded by the first virus (i.e. tobacco mosaic virus), where the multiplication of the second virus (i.e. aucuba mosaic virus) is inhibited by the pre-existence of the first one.

The centers of aucuba mosaic multiplication will therefore form islands in an area more and more invaded by the „premunizing" virus. Since the tissues infected by the virus of the common tobacco mosaic do not in general acquire a chlorotic aspect in leaves of Solanum Seaforthianum, the small yellow islands, consisting of cells containing tobacco mosaic virus, stand out against the normal green area. The aucuba mosaic virus might establish itself in these yellow islands and spread to a certain extent, this depending on the degree of premunity. It is also likely that in some circular zones invaded by aucuba mosaic virus the multiplication of this virus is not entirely suppressed, but only slowed down by the occurrence of very small quantities of tobacco mosaic virus in some cells. This would explain, why in some cases the chlorotic ring remains narrow, since, (according to KOEHLER's theory), the periodic stimulus inducing necrotization of the cells situated on the periphery of the ring lesions would affect them before

the virus had had time to infect the large circular regions. We can also understand that the periphery of the spots sometimes differs considerably from a circle, since the centrifugal spread of the aucuba mosaic virus may be inhibited by the pre-existing tobacco mosaic virus more in one sector than in another. In plants which have not been „premunized", the leaf becomes, however, circular invaded by the aucuba mosaic virus in progressively larger zones, which finally render the whole leaf surface almost completely chlorotic.

SUMMARY

1. A comparative study has been made of the symptoms induced respectively by the tobacco mosaic and by the aucuba mosaic virus on leaves of *Solanum Seaforthianum.*
2. Whereas leaves inoculated with sap containing tobacco mosaic virus do not normally exhibit any primary symptoms, aucuba mosaic virus induces the formation of a great number of ring spot lesions; these lesions increase with time, gradually conferring a chlorotic aspect to a great part of the leaf surface. The secondary symptoms of aucuba mosaic of *Solanum Seaforthianum* consist largely of isolated yellowish-white ring lesions, while secondary mosaic symptoms are limited to diffuse veinbanding, malformation, and twisting of the young leaves.
3. Leaves incompletely „premunized" with tobacco mosaic virus and locally superinoculated with aucuba mosaic virus exhibit small, conspicuous ring spot symptoms (or small yellow disks), which stand out owing to their clear-cut borderline against the dark green remainder of the leaf surface. In corresponding leaves of plants which have not been „premunized" the aucuba mosaic-induced ring spot lesions are larger, more diffuse and often without any well defined borderline. Some time after inoculation, the difference between these ring spot lesions and the originally unaffected part of the leaf area becomes scarcely noticeable in such leaves.
4. In leaves of *Solanum Seaforthianum* systemically „premunized" against aucuba mosaic, the ring spot lesions remain small and stand out against the dark green of the rest of the leaf blade, owing to their clear-cut borderline and clear color.
5. In the discussion we try to relate the type of ring spot lesion in „premunized" leaves to the presumptive manner of virus multiplication in leaves previously infected with a virus strain antagonistic in its effect to that of the virus used in the superinoculation.

SAMENVATTING

1. Een vergelijkend onderzoek werd ingesteld naar de symptomen veroorzaakt door de viren van het gewone tabaksmozaïek en het aucubamozaïek op bladeren van *Solanum Seaforthianum.*
2. Terwijl bladeren, die geinoculeerd zijn met sap, dat tabaksmozaïekvirus bevat, normaliter geen primaire symptomen vertonen, geeft aucubamozaïekvirus aanleiding tot de vorming van een groot aantal ringvormige vlekken („ringspot"). Deze vlekken worden langzamerhand groter en geven aan een groot gedeelte van het bladoppervlak een chlorotisch uiterlijk. De secundaire symptomen

van aucubamozaïek op *S. Seaforthianum* bestaan grotendeels uit geisoleerde geelwitte ringen, terwijl de secundaire mozaïeksymptomen zich bepalen tot diffuse donkerder groene verkleuring langs de nerven („veinbanding") en misvorming van de jonge bladeren.

3. Bladeren, die niet volkomen besmet zijn door het tabaksmozaïekvirus en die plaatselijk voor de tweede maal geinoculeerd zijn met het aucubamozaïekvirus vertonen kleine duidelijke ringspotsymptomen, die opvallen als gevolg van de rand, die scherp afsteekt tegen de donkergroene omgeving van het gezonde bladoppervlak. In overeenkomstige bladeren van planten, die niet gepremuniseerd zijn, zijn de vlekken („ringspot"), te weeg gebracht door het aucubamozaïekvirus, groter, meer diffuus en dikwijls zonder een duidelijke rand. Enige tijd na de inoculatie is in zulke bladeren het verschil tussen de ringvormige vlekken en het oorspronkelijk niet aangetaste deel van het bladoppervlak nauwelijks meer waar te nemen.
4. In bladeren van *S. Seaforthianum*, die een „systemic premunisation" hebben ondergaan tegen het aucubamozaïek, blijven de ringvormige vlekken klein en steken zij duidelijk af tegen het groen van het blad als gevolg van hun scherpe rand en de lichte kleur.
5. In de discussie wordt getracht verband te leggen tussen het type van de ringspot-symptomen in gepremuniseerde bladeren en de wijze, waarop de vermeerdering van het virus vermoedelijk plaats vindt in bladeren, die vooraf geinfecteerd zijn met een virusstam, die antagonistisch is ten opzichte van het later geinoculeerde virus.

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