ACTA BOT. CROAT. VOL. 40, 7—18, 1981.

UDC 58
CODEN: ABCRA2
YU ISSN 0365—0588

UDC 582.951.4 : 576.858.8/576.8.097.31 = 20

EFFECT OF SENESCENCE ON LESION FORMATION AND THE LEVEL OF INDUCED RESISTANCE IN SAMSUN NN PLANTS INFECTED WITH TMV. I. EXPERIMENT WITH CHANGING PHOTOPERIOD

KAZIMIERZ A. MICZYŃSKI

(Institute of Plant Biology, Academy of Agriculture, Kraków, Poland)

Received December 12, 1980

Introduction

The theory of acquired or induced resistance, first formulated in 1961 by Ross and his coworkers (Bozarth and Ross 1964, Ross 1961 a, b) assumes that virus infected plants develop in tissues and organs not invaded with the infectious agent, a certain level of resistance against challenge infections with the same virus or even some other types of viruses. The intensity of this defence reaction can be most easily estimated in hypersensitive hosts, e. g. in the virus — host combination TMV and Samsun NN tobbacco, which forms necrotic lesions at the sites of virus penetration into the infected leaf. The virus becomes localised in such lesions due to a strong resistance, which develops in surroundig cells, preventing both further expansion of the virus, and restraining its multiplication. Moreover, the adjacent tissues become resistant to superinfection (secondary or challenge infection) with the virus, and this was called by Ross "local acquired resistance" (Ross 1961 a). After a few days this resistance spreads all over the plant and can be detected even in the parts most remote from the original site of infection, which have not been invaded by the virus (Ross 1966).

Activation of anti-viral resistance mechanisms in virus infected leaves of hypersensitive hosts results among others in the reduction of the number or size (or both number and size) of necrotic lesions formed after challenge inoculation. The comparison of those values with the appropriate values

for control leaves (which were not infected previously), provides an indirect measure of the intensity of induced resistance. To estimate the increase of resistance in the hypersensitive leaf, induced by a primary virus infection, we can use the following formula: % of induced resistance $= 100 - \frac{T}{C} \cdot 100$, in which T denotes average value of lesion characteristics (size, number, necrotised area) for leaves with induced resistance, and C comparable values for lesions formed on control leaves. The calculated value says by what percentage, relative to control plants, the appropriate lesion characteristic of plants with induced resistance was reduced (Ross 1966). For plants, having fairly large leaf blades, the most direct comparison of the effects of induced resistance can be made by the half-leaf method (Ross 1961 a, b). Primary, or resistance-inducing infection is applied to the one half of the leaf, whereas challenge inoculation to the other (opposite) half, after an appropriate time (Fig. 1). Lesions formed on that second half-leaf are then compared with those which arise on simultaneously infected control plants.

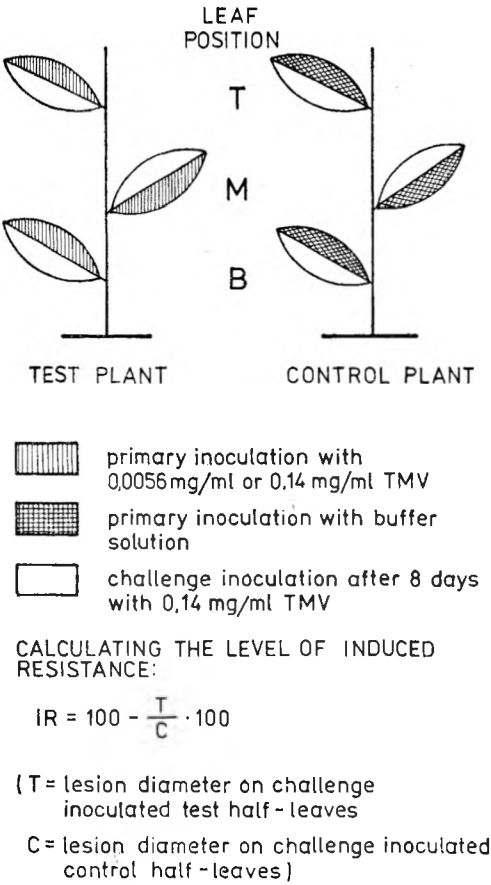

Fig. 1. Method of inducing resistance.

In normal greenhouse conditions (temp. ca. 20° C), necrotic lesions formed on TMV infected leaves of tobacco Samsun NN, appear already on the 2nd or 3rd day after infection. During subsequent 5—6 days they increase in size, the rate of this increase as well as their final dimensions being dependent on both the external conditions of plant growth and the physiological state of the infected leaf. It is assumed that the size of lesions is proportional to the amount of virus material which can be extracted thereof. Hence it can be regarded as the best index of the intensity of viral multiplication in the infected leaf (Sela et al. 1969). For the same reason lesion size provides a good measure for both the local and systemic resistance, induced by TMV infection in hypersensitive tobacco varieties, — contrary to lesion numbers.

As was pointed out by several authors (Ross 1966, Simons and Ross 1971), the number of lesions, formed on challenge inoculated leaves of those varieties reveals a great variability which cannot always be correlated with the level of induced resistance. For instance, using a high concentrated TMV inocula for challenge inoculation one usually obtains more lesions on "resistant" leaves, than on control ones (Simons and Ross 1971). Therefore in those virus-host combinations lesion numbers cannot be used for measuring the level of induced resistance.

This interesting feature of the physiology of resistance in the plants mentioned seems to be correlated with the age of the infected leaf. According to the relation usually observed between lesion numbers and leaf position in TMV infected hypersensitive plants, leaves situated lower on the stem develop usually more lesions per sq. cm, than the upper ones until their growth is stopped and they begin to age (Kiraly et al. 1968, Miczyński 1970). On the other hand it is well known, that ageing of plants usually increases their resistance against virus invasion (Gaboryanyi and El Hammady 1969, Nienhaus 1973, Sela et al. 1969). In Samsun NN necrotic lesions which are formed on older leaves, sooner stop their expansion, and are usually smaller, than those which arise on young leaves (Bozarth and Ross 1964). Their average dimensions increase gradually from the base to the apex of the infected plant. A similar trend was observed by Ross and his coworkers in the development of systemic acquired resistance in TMV infected Samsun NN (Balazs and Gaboryanyi 1974, Ross 1961 b, 1966). After infection of half-leaves of that plant, situated at different positions on the stem, the highest level of induced resistance in opposite halves was found in the oldest leaves, whereas the lowest level was developed in the upper leaves. Thus both types of resistance: local and systemic were expressed much more strongly in the older leaves than in the young ones. It is assumed therefore, that in both cases the same mechanisms of resistance are involved. According to such supposition the same metabolic processes which are responsible for the localisation of the virus at the infection site produce in the whole plant a status of resistance which reduces to a greater extent virus multiplication in case of challenge inoculation. Induction of acquired resistance thus reflects intensification of normal defense reactions, which prevent the virus spread in the hypersensitive host (Simons and Ross 1971). Hence, as was shown by several authors, the same exo- or endogenous factors cause similar changes in the appearance of both primary and secondary lesions (Bozarth and Ross 1964, Gaboryanyi and El Hammady 1969, Gaboryanyi et al. 1969, Nienhaus 1973, Ross 1966).

The above observations are to a certain extent inconsistent with the results of those experiments in which the process of senescence of to-

bacco leaves was artificially stimulated by treatments with different chemicals or by separation from the plant (Kiraly et al. 1968, Loebenstein et al. 1969, Nakagaki and Matsui 1971, Nakagaki and Hirai 1971). In such cases often an increase of virus concentration in infected leaves was observed, which could be interpreted as a consequence of a decreased resistance of the treated tissues. It must be noted however, that in all those experiments the treated leaves were subjected to treatments specificially affecting certain links in their metabolism, and this was probably the cause of the discrepancies observed in the results in both types of experiments.

In order to obtain a clear insight into the role of senescence in the development of anti-TMV resistance in leaves of hypersensitive tobacco it was necessary to stimulate this process in a most natural manner, uniformly in the whole plant, and to see how it affects the level of resistance induced by TMV. In the present study the author stimulated senescense of the tobacco plants investigated (cv. Samsun NN), raising them under unfavourable conditions of a short day — 9 hrs. As is well known the lack of light stimulates leaf senescence which sooner becomes yellow and decay. The use of this factor secured stimulation of that process in a manner most similar to the natural.

Material and Methods

Tobacco plants of the variety Samsun NN were raised in the greenhouse until they attained the stage of 3—4 well developed leaves. They were then transferred to growth rooms with constant temperature of ca. 20° C and light of 3600 lux on the bench level (fluorescent and incandescent lamps). The plants were devided into 2 series, each consisting of 3 experimental groups: 2 test groups and 1 control group. Each series was placed into a separate growth-room with 9 and 16 hr. photoperiods, respectively. After 2 days following the transfer to the growth chambers all plants in the test groups were inoculated with TMV alternatively on right or left halves of three leaves situated on 3 neighbouring stem nodes (primary inoculation) (Fig. 1). The corresponding half-leaves on control plants were rubbed with buffer solution used for preparation of virus inoculum. Plants of one test group in each series were infected with 0.14 mg/ml TMV (tomato strain) solution, and in the second group with a more diluted solution of 0.005 mg/ml. Thus both test groups represented two different levels of induced resistance. On the 8th day, following the first inoculation, the opposite half-leaves on each plant were challenge inoculated with 0.14 mg/ml solution of the same virus. The number of lesions on primary, and both number and sizes on challenge (secondary) inoculated half-leaves were estimated 7 days later, and the appropriate levels (percentages) of induced resistance were calculated.

Results

The photoperiods applied affected differently the growth of the tobaccos investigated. Plants raised at a 9 hr. day were growing slowly, and their leaves after infection with TMV turned yellow so quickly, that it was impossible to examine lesions at the lowest leaf position. Therefore only the results from the middle and top leaves were taken for comparisons.

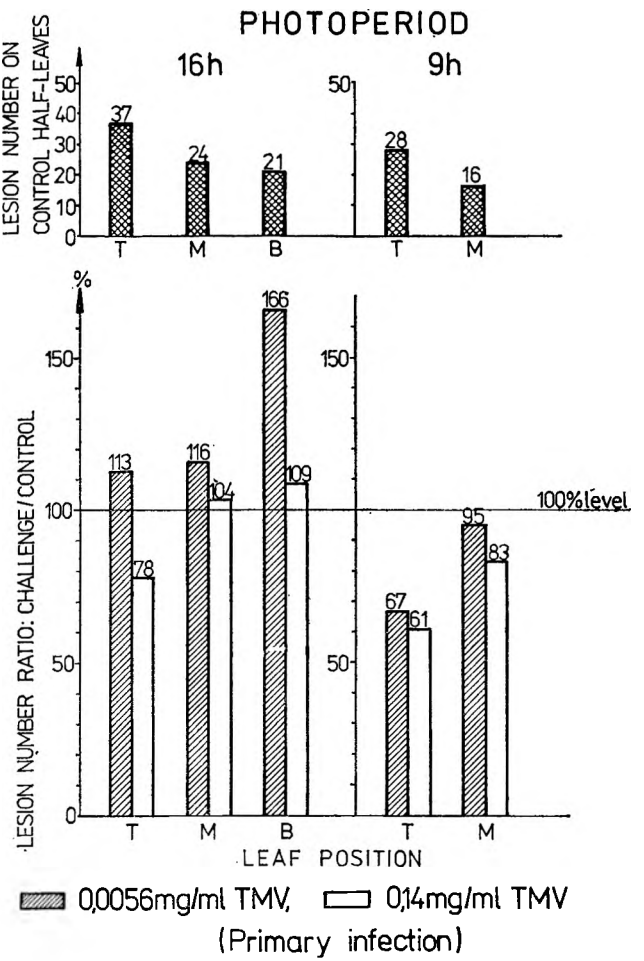

Fig. 2. Effect of photoperiod and the concentration of primary TMV inoculum on the number of lesions on challenge TMV infected half-leaves of Samsun NN tobacco; expressed as ratios to lesion numbers on comparable control half-leaves.

Lesion counts, obtained in one typical experiment are presented in Table 1. As seen, in plants kept at a 16 hr. day the susceptibility of leaves to primary TMV infection increased from the top to the base of the plant. There were on an average: 6, 9, and 14 lesions formed after inoculation with 0.0056 mg/ml TMV, on the top, middle and bottom half-leaves respectively, or 45, 75 and 85 lesions after inoculation with 14 mg/ml TMV. Eight days later, at the moment of challenge inoculation, highest susceptibility to TMV was shifted to the upper leaves (control plants), and the same tendency appeared in challenge inoculated leaves with induced resistance, but only in those which were previously infected with a more diluted TMV preparation.

In plants grown at a 9 hr. photoperiod there were no differences in susceptibility to primary TMV infection between leaves at different positions on the stem. Such difference appeared 8 days later, the top leaves becoming also here more susceptible (control group). Induction or resistance by previous infection of the opposite half-leaf abolished however this differences in both test groups of plants.

An interesting illustration of the effect of the intensity of primary TMV infection on the number of lesions on challenge inoculated half-leaves is presented in Fig. 2 and is based on the data in Tab. 1. As can be seen, in plants cultivated on a 16 hr. day the number of secondary lesions, formed on leaves previously infected with a lower concentration of TMV, exceeded visibly the corresponding lesion numbers in control leaves. Those numbers were however considerably reduced in leaves with a higher level of induced resistance (infected previously with 0.14 mg/ml TMV). In plants cultivated on a 9 hr. photoperiod, primary

Table 1. Effect of changing photoperiod on the numbers of necrotic lesions on leaves of tobacco Samsun NN after primary or challenge infection with TMV.

Photoperiod hrs	Experimental groups	Average lesion number on half-leaf	
		Primary	Secondary
		T M B	T M B
16	Control	— — —	37 24 21
	0.0056 mg TMV	6 9 14	42 28 35
	0.14 mg TMV	45 75 85	29 25 23 n
9	Control	— — —	28 16 —
	0.0056 mg TMV	19 13 — n	19 15 — n
	0.14 mg TMV	54 58 — n	17 13 — n

Explanations: T — top leaf, M — medium leaf, B — basal leaf, »n« — differences between numbers in the row insignificant.

infection of half-leaves with either virus concentration resulted in a considerable reduction of lesion numbers on the challenge inoculated halves as compared with the control. Also, the differences in secondary lesion numbers between plants infected primarily with different TMV concentrations, appeared insignificant here.

It seems reasonable to regard those relationships as a result of an activation of resistance mechanisms against TMV in plants cultivated under unfavourable conditions of a short photoperiod. Higher levels of induced resistance in those plants become still more obvious when appropriate data on lesion sizes are compared (Tab. 2 and Fig. 3). The difference between the series cultivated on 16 hour or 9 hour day appeared at both levels of induced resistance, but was evidently smaller in plants infected previously with a higher concentration of TMV (left side of Fig. 3). Treatment with a short photoperiod, however reduced, the difference between the two levels of acquired resistance, induced by previous infection with either 0.14 or 0.0056 mg/ml TMV (right side of the Fig. 3*).

* Data from Tab. 2 are used in Fig 3.

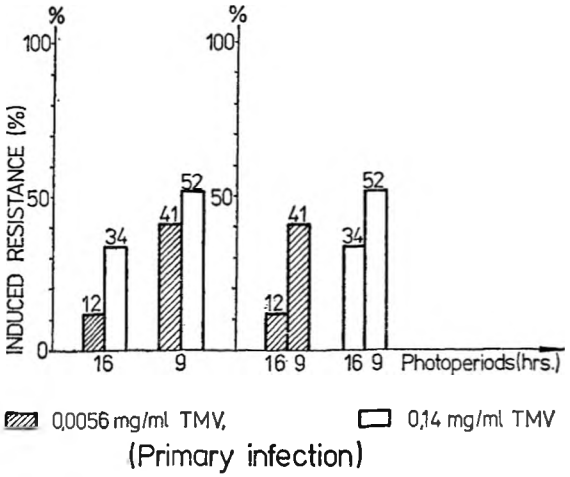

Fig. 3. Effect of photoperiod on the level of TMV resistance in leaves of Samsun NN tobacco; induced after inoculation with various concentrations of this virus.

Table 2. Average size of secondary lesions formed after TMV infection of Samsun NN plants cultivated at different photoperiods.

Photoperiod	Experimental series	Average lesion diameter (mm)			
		Leaf position			Average (T + M)
		Top	Medium	Basal	
16 hours	Control (primary inoculation with water)	0.94	0.99	1.00	0.97
	Primary infection with 0.056 mg ml TMV	0.84	0.85	0.73	0.85
	Primary infection with 0.14 mg ml TMV	0.67	0.60	0.62	0.63
9 hours	Control (primary inoculation with water)	0.99	0.92	—	0.96
	Primary infection with 0.056 mg ml TMV	0.55	0.59	—	0.57
	Primary infection with 0.14 mg/ml TMV	0.45	0.46	—	0.46

Explanation: the last column presents averages from top and medium leaves.

Contrary to the secondary lesion numbers, there were no significant differences in their sizes between the leaves occupying different positions on the stem (Tab. 2). Nevertheless in plants raised on a 16 hr. day the

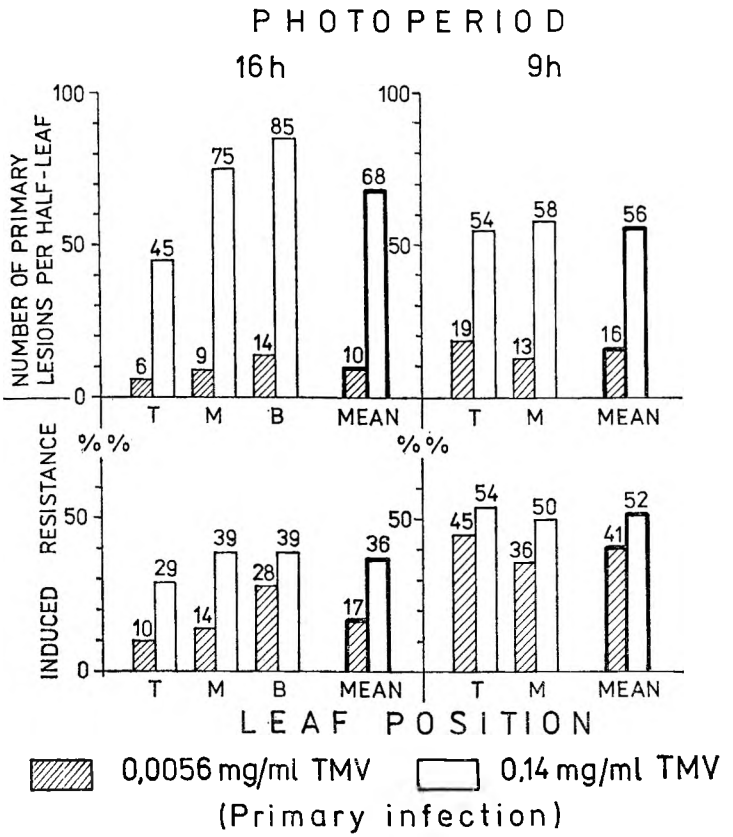

Fig. 4. Effect of photoperiod and the intensity of primary infection on the level of induced resistance; expressed as percentage reduction of lesion sizes after challenge inoculation with TMV.

lower leaves developed a significantly higher level of induced resistance than the upper ones. These differences were more conspicuous in plants to which a more diluted virus preparation was applied as a resistance inducing agent (Fig. 4). In the same experimental series the smallest differences in resistance induced by both virus concentrations were found in the basal leaves. One can also see that the discussed levels of acquired resistance at different leaf positions were closely correlated with the numbers of primary lesions on opposite half-leaves, — that is — with the intensity of the resistance inducing stimulus. Higher levels of that resistance were developed by those leaves on which more lesions appeared upon primary infection.

In plants raised at a 9 hr. photoperiod no significant differences in levels of induced resistance occured between the leaves at the upper and the middle positions on the stem.

Summarising the data presented it can be said, that at given experimental conditions there is a close correlation between the suspectibility of Samsun NN leaves to the infection with TMV, and their ability to

develop systemic resistance to challenge inoculation with the same virus. This correlation can be modified by changing the external conditions at which the plants are cultivated. Older leaves, which usually are more susceptible to viral infection (Balazs and Gaboryanyi 1974, Balazs et. al. 1973), develop much more intense defense reactions which are reflected by a greater reduction of lesion sizes after challenge inoculation. Raising plants in unfavourable light conditions stimulates their senescence, which in turn activates the defense reactions against TMV in particular leaves, but at the same time reduces differences between them in that respect.

The use of arithmetical means of lesion sizes for the evaluation of the levels of induced resistance in TMV infected leaves of Samsun NN does not provide information on the dynamics of their development, which is one of essential features of the intensity of induced defense reactions in the leaf. Investigations carried out by Simons and Ross (1971) have shown, that necrotic lesions formed in this system after challenge infection appear much quicker but also sooner stop their enlargement, than those arising on the control (non induced) plants (Simons and Ross 1971). In consequence, different frequency distribution curves of lesion sizes are obtained for those two types of TMV infected leaves (Ross 1966). In the control leaves this distribution is more or less normal, whereas in "resistant" leaves it is distinctly skew with its modal value shifted towards the diminishing lesion diameters (Fig. 5). The distance between the modal and median values provides a highly precise measure of the level of induced resistance, and can be simultaneously used for the evaluation of leaf resistance to primary infection. In this aspect it provides a more universal measure of anti-viral resistance of a local lesion host, than the comparison of lesion sizes. Fig. 5 shows frequency distribution curves of lesion sizes designed from data of the experiments described above. As seen, — the short photoperiod induced a certain level of resistance to

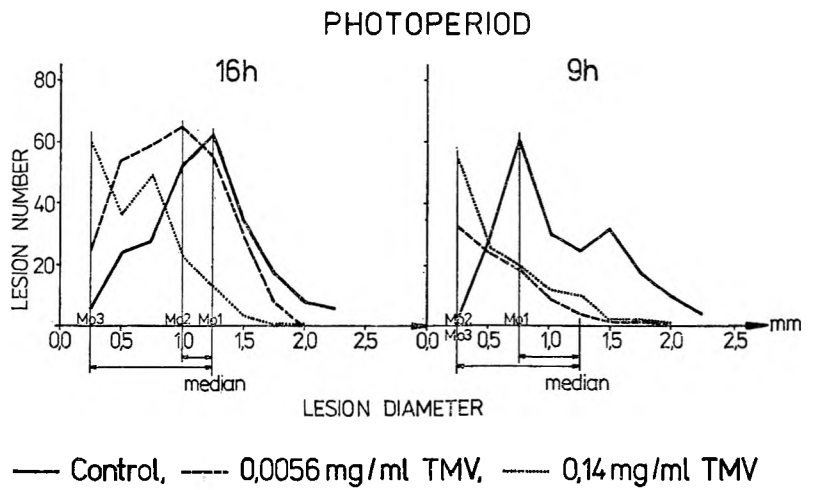

Fig. 5. Effect of photoperiod and the intensity of primary infection on the size distribution of lesions on top leaves; challenge inoculated with TMV.

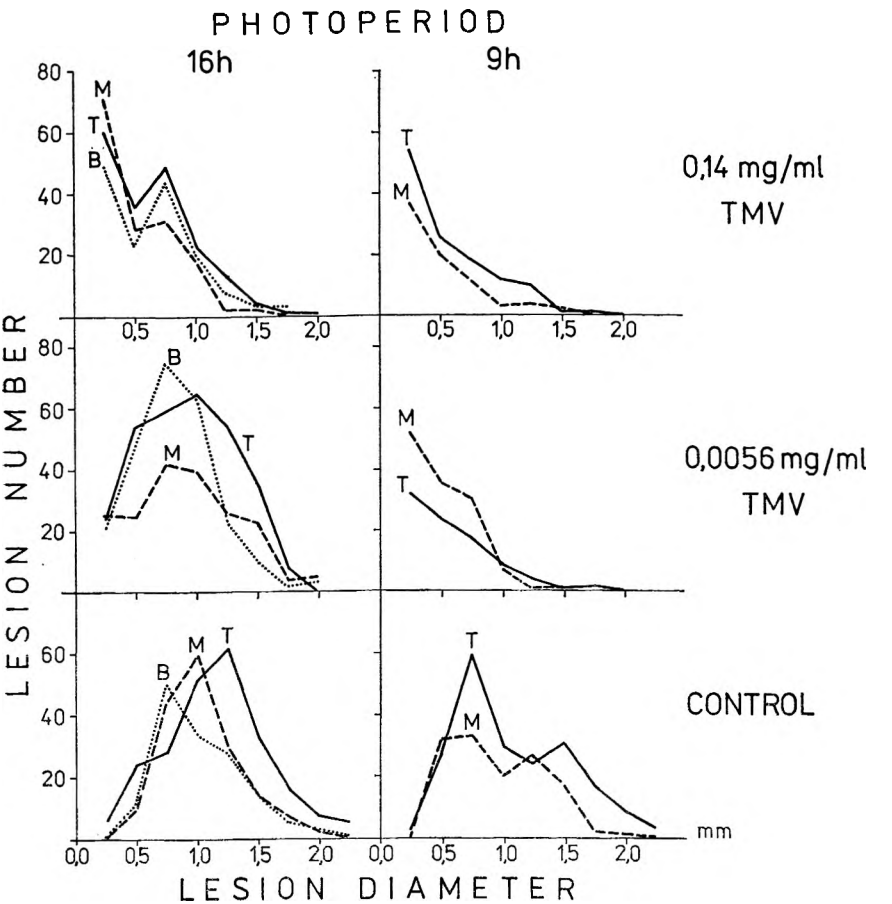

Fig. 6. Effect of photoperiod and leaf age on the size distribution of lesions induced by TMV in leaves previously infected with various concentrations of the virus.

TMV already in the control plants. Infection with this virus considerably increased this resistance in the "short-day" plants which evidently surpassed the resistance levels of similarly treated plants, grown at a longer photoperiod.

Similar frequency distribution curves, calculated for separate leaf positions are shown in Fig. 6. It can be seen, that for plants grown at a long day of 16 hr. those curves are skew for basal leaves even in the control group, this skewness being subsequently induced by primary virus infection also at higher leaf positions. In plants, grown at a short day both the upper and the medium leaves show a still higher degree of skewness of their frequency distribution curves, — both in the control and in the two test plant groups. In the latter, induction of resistance by primary virus infection resulted in a shift of the respective modal to minimal values of lesion sizes which were still discernible by the techniques applied.

Discussion

It seems obvious that frequency distribution curves of lesion sizes enable us to detect very subtle differences in the resistance between particular leaves which could be induced not only by viral infection but also by other factors affecting the process of plant ageing. The concurrent shapers of these curves, resulting either from a previous viral infection or from the natural senescence of the leaf indicate clearly that the process of leaf senescence is stimulated by viral infection. Older leaves in spite of their higher susceptibility to TMV reveal, as a rule, a lower ability for the differentiation of the changing concentrations of this virus in the local lesion test. This certainly results from a higher level of resistance against TMV multiplication in those leaves.

Summary

Inoculation of Samsun NN tobacco leaves with TMV results in a hypersensitive reaction so that local necrotic lesions appear. Afterwards the whole plant reaches a status of resistance to superinfection with the same virus. This specific resistance manifests itself in the reduction of the size of necrotic lesions arising on the leaf after superinfection.

The level of specific resistance depends on many different factors, among which on the age of the infected plant or even of particular leaves. Older leaves are usually more resistant. In the present study the author stimulated the natural process of senescence by raising the plants under unfavourable conditions of a short photoperiod. It was shown that the plants influenced by a short photoperiod developed, after TMV inoculation, a much higher level of induced resistance.

The number of lesions is not a good index of the level of induced resistance. The results show that a good index of the level of induced resistance can be provided by modal values of the frequency distribution curves of lesion diameters. They are a better index than the arithmetic means because they also give an idea on the dynamics of lesion growth.

References

Balazs, E., R. Gaboryanyi, 1974: Ethrel induced leaf senescence and increased TMV susceptibility in tobacco. Z. Pfl. Krankh. Pflschutz. *81,* 389—393.

Balazs, E., R. Gaboryanyi, Z. Kiraly, 1973: Leaf senescence and increased virus susceptibility in tobacco: the effect of abscissic acid. Physiol. Plant Path. *3,* 341—346.

Bozarth, R. F., A. F. Ross, 1964: Systemic resistance induced by localised virus infections: Extent of changes in uninfected plant parts. Virology *24,* 446—451.

Gaboryanyi, R., M. El Hammady, 1969: Effect of temperature on size of local lesions by TMV on tobacco plants. Acta Phytopath. Hung. *4,* 125—129.

Gaboryanyi, R., E. Balazs, Z. Kiraly, 1969: Ethylene production, tissue senescence and local virus infections. Acta Phytopath. Hung. *4,* 355—358.

Kiraly, Z., M. El Hammady, B. J. Pozsar, 1968: Susceptibility to tobacco mosaic virus in relation to RNA and protein synthesis in tobacco and bean plants. Phytopath. Z. *63,* 47—63.

Loebenstein, G. B., T. Sela, T. van Praagh, 1969: Increased tobacco mosaic local lesion size and virus multiplication in hypersensiitve hosts in the presence of actinomycin D. Virology *37,* 42—48.

Miczyński, K. A., 1970: Susceptibility of tobacco leaves of different age to the infection with the potato virus X (PVX) and its nucleic acid (PVX-RNA). Z. Probl. Post. Nauk Roln. *111*, 111—120.
Nakagaki, Y., C. Matsui, 1971: Effect of bean-leaf detachement on susceptibility to tobacco mosaic infection. Phytopathology *61*, 354—356.
Nakagaki, Y., T. Hirai, 1971: Effect of detached leaf treatment on tobacco mosaic virus multiplication in tobacco and bean leaves. Phytopathology *61*, 22—27.
Nienhaus, F., 1973: Einfluss von Alterungsprozessen auf die nekrotische Reaktion von Pflanzen nach Virusinfektion. I. Pflanzenalter, Spross und Blattabtrennung. Z. Pfl. Krankh. u. Pflschutz. *80*, 418—423.
Ross, A. F., 1961a: Localized acquired resistance to plant virus infections in hypersensitive hosts. Virology *14*, 329—339.
Ross, A. F., 1961 : Systemic acquired resistance induced by localised virus infections in plants. Vrology *14*, 340—358.
Ross, A. F., 1966: Systemic effects of local lesion formation. Viruses of Plants, Amsterdam, 127—150.
Sela, B., G. Loebenstein, T. van Praagh, 1969: Increase of virus multiplication and lesion size in hypersensitive hosts in the presence of chloramphenicol. Virology *39*, 260—264.
Simons, T. J., A. F. Ross, 1971: Metabolic changes associated with systemic induced resistance to tobacco mosaic virus in Samsun NN tobacco. Phytopathology *61*, 293—300.

SAŽETAK

UČINAK STARENJA NA STVARANJE LEZIJA I STUPANJ INDUCIRANE OTPORNOSTI U DUHANU SAMSUNU NN INFICIRANOM S VIRUSOM MOZAIKA DUHANA. I. POKUS PROMJENE FOTOPERIODA

Kazimierz A. Miczyński

(Institut za biologiju biljaka, Poljoprivredna akademija, Kraków, Poljska)

Inokulacija duhana samsuna NN virusom mozaika duhana izaziva hipersenzitivnu reakciju, tako da na listovima nastaju mnogobrojne lokalne nekrotične lezije. Poslije toga biljka postiže stanje otpornosti protiv superinfekcije s istim virusom. Ta specifična otpornost očituje se u redukciji veličine nekrotičnih lezija, koje se pojave na listovima poslije superinfekcije.

Stupanj specifične otpornosti ovisi o mnogim različitim faktorima, među ostalim i o starosti inficirane biljke ili čak pojedinih listova. Stariji su listovi obično otporniji. U ovoj studiji autor je pokazao kako je utjecao na prirodni proces starenja uzgajajući biljke pod nepovoljnim uvjetima kratkoga dana. Pokazalo se da su te biljke pod utjecajem kratke foto-periode dostizale poslije inokulacije virusom mozaika duhana mnogo veći stupanj inducirane otpornosti.

Broj lezija nije dobar indeks za stupanj inducirane otpornosti. Ovdje izneseni rezultati pokazuju da modalne vrijednosti čestoće distribucijskih krivulja dijametara lezija daju dobar indeks za stupanj inducirane otpornosti. One su bolji indeks nego aritmetičke sredine jer upozoravaju također i na dinamiku rasta lezija.

Dr. Kazimierz Miczyński
Akademia Rolnicza
Al. 29 Listopada 48
Kraków, Polska