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EFFECT OF SENESCENCE ON LESION FORMATION AND THE LEVEL OF INDUCED RESISTANCE IN SAMSUN NN PLANTS INFECTED WITH TMV. II. TOPOGRAPHY OF TMV RESISTANCE IN SAMSUN NN LEAVES

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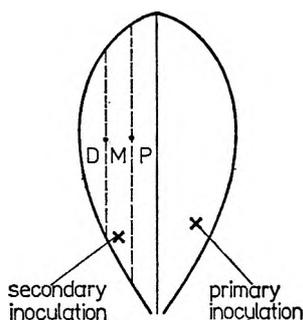
Introduction

The second part of this investigation was devoted to the study of the topography of TMV resistance within the leaf blade. It was shown by Avery (1933), that tobacco leaf is not physiologically uniform over its whole area. The apical and central parts of the leaf-blade are older than its marginal and basal parts, as they sooner loose their meristematic activity. While measuring TMV lesions on Samsun NN leaves, a great variation of their sizes even within the same leaf was stated. It was therefore interesting to see to what extent this variation reflected the natural physiological differentiation of the leaf blade, and to what degree it could be changed in plants in which additional resistance was induced by previous infection with TMV.

Material and Methods

Experiments were performed with the same tobacco variety Samsun NN, on plants having 6—8 well developed leaves. They were likewise kept in a growth room, but this time only at a 16 hr photoperiod. 24 hr prior to virus infection they were topped and trimmed to 3 leaves, situated on three consecutive stem nodes, in order to reduce the different susceptibility of leaves to virus infection. The plants were divided into five

groups: a control one and 4 test groups, each of those four being subsequently inoculated with a different concentration of TMV, according to the half-leaf inoculation scheme described previously (Miczyński 1981). Eight days later, the opposite half-leaves were challenge inoculated, together with the half-leaves of control plants, with a 60 g/ml solution of the same virus. Lesion assays were performed on the 7th day following the challenge infection, by measuring both their density and size on particular leaves. Before the assays, the challenge inoculated half-leaves were divided parallelly to the main vein into three zones of equal width (as measured at the widest spot of the blade) (Fig. 1), and the respective



P-proximal, M-medial, D-distal

Fig. 1. Inoculation scheme and the allotted leaf zones.

assays were made separately in each zone. It was assumed that the zones: proximal, medium and distal in respect to the main vein, roughly reflected the natural differences in the physiological age of different parts of the leaf, as described by A very (1933, 1934, 1935), the proximal being the oldest and the distal the comparatively youngest of the designed zones. Levels of induced resistance were calculated then for each zone by the formula described previously.

Results

Variability of primary lesions

In order to test to what extent the applied trimming of plants was really successful in reducing variability of leaves in their susceptibility to TMV, parallelly to the control series additional 6 untrimmed plants were inoculated with the virus. It was stated that necrotic lesions on trimmed plants were slightly more uniform in size, but at the same time also much bigger, than those formed on untrimmed plants (Tab. 1). Average lesion diameters on the top, middle and basal leaves on untrimmed plants were: 1.25, 1.0, and 1.0 mm, whereas on trimmed plants they amounted to: 1.55, 1.74, and 1.65 mm respectively. The treatment applied did not eliminate the greater susceptibility of the lower leaves to infection with TMV as compared with the upper ones, which was visible at different concentrations of the inocula applied.

Table 1. Effect of trimming on the variability of necrotic lesions, formed on Samsun NN leaves upon infection with TMV

| Average lesion diameter (mm) | Trimmed plants | | | Untrimmed plants | | |
|--------------------------------------------------|----------------|--------|--------|------------------|--------|--------|
| | Leaf position | | | | | |
| | top | middle | bottom | top | middle | bottom |
| | 1.55 | 1.74 | 1.65 | 1.25 | 1.00 | 1.01 |
| Average number of primary lesions per 100 sq. cm | | | | | | |
| concentration of primary inoculum, µg/ml TMV | | | | | | |
| 0.0 (control) | — | — | — | | | |
| 0.3 | 5 | 9 | 13 | | | |
| 3.0 | 20 | 16 | 39 | | | |
| 30.0 | 29 | 40 | 83 | | | |
| 60.0 | 130 | 127 | 180 | | | |

In order to see whether the allotted leaf-zones differed in their susceptibility to TMV infection, lesion numbers in each zone were recalculated to lesion densities, and in this manner comparable values were obtained (Tab. 2). As seen, the susceptibility of Samsun NN leaves to primary TMV infection was not uniform over their whole area. On top and medium leaves it was highest in the proximal zone, and lowest in the distal zone, whereas on bottom leaves the situation was quite opposite (Tab. 2. column 1). There was also a considerable variation in lesion sizes. In all leaves much larger lesions were formed in the marginal area of the leaf-blade, than in its central zone. This differentiation was larger on the basal leaves, than on the upper ones (Tab. 2, column 2). The total necrotic area, calculated from lesion size and numbers (16), was distributed almost uniformly among all the three zones on the top leaves, whereas on basal and medium leaves it was on the average 3 and 2 times larger in the distal zone as compared with the proximal part (Tab. 2, column 3). Such unequal necrotisation of the leaf-blade in particular zones was at least partially due to a similarly unequal distribution of the smallest measurable lesions of the diameter up to 0.5 mm. Prevailing numbers of those microlesions were formed in the proximal zones of all leaves compared (Tab. 2, column 4). Their distribution was, however, also more uniform in the upper leaves, a comparatively higher percentage of those lesions being formed in the distal zone of the upper leaves, than in the same zone of the basal leaves.

Variability of secondary lesions

Table 3 presents data on the effect on the intensity of primary infection with TMV on the scope of necrotic reactions in opposite half-leaves of Samsun NN, infected 8 days later with the same virus. It shows as well known decrease in secondary lesion diameters, proceeding parallelly with the increase in concentration of primary inoculum, that is with the increase in the level of induced resistance. It should be noticed, that average lesion diameters on control plants were slightly larger on the basal and medium leaves, than on the top ones, whereas in plants, infected previously with 60 µg/ml TMV — they were smaller on the basal leaves in comparison

Table 2. Necrotic reactions in different zones of Samsun NN tobacco leaves after infection with 60 µg/ml TMV (Control series).

| Leaf zone | Number of lesions % 1). | Average lesion diameter mm | | | | | | Participation in necrotized area (% 2). | Number of micro-lesions (diameter up to 0,5 mm ³ 3). | | | | | |
|------------------|----------------------------|-------------------------------|--------|-------|------|------|--------|--------------------------------------------|-----------------------------------------------------------------------|--------|-------|------|-------|----|
| | | Leaf position | | | | | | | top | medial | basal | mean | | |
| | | top | medial | basal | mean | top | medial | | | | | | basal | |
| Proximal | 136 | 1.47 | 1.27 | 1.17 | 1.34 | 1.06 | 34 | 20 | 17 | 24 | 29 | 37 | 36 | 33 |
| Medium | 121 | 1.42 | 1.77 | 1.72 | 1.61 | 30 | 34 | 32 | 32 | 34 | 34 | 22 | 10 | 24 |
| Distal | 100 | 1.83 | 2.29 | 1.95 | 2.02 | 36 | 46 | 51 | 44 | 18 | 2 | 9 | 10 | |
| dist/prox. ratio | | 1.24 | 1.81 | 1.67 | 1.50 | 1.06 | 2.3 | 3.0 | 1.8 | | | | | |

Explanations: 1) Expressed in percentage of lesion number in the distal zone. 2) Expressed in percentage of total necrotic area on the half-leaf. 3) Expressed in percentage of lesion totals in each zone.

Table 3. Effect of increasing concentration of primary TMV inoculum on the number and diameter of secondary lesions formed on opposite half-leaves of Samsun NN upon infection 8 days later with 60 $\mu\text{g/ml}$ TMV

| Concentration of primary inoculum $\mu\text{g/ml}$ TMV | Average lesion diameter mm | | | | Average lesion number per 100 sq. cm | | | |
|--------------------------------------------------------|----------------------------|--------|------|------|--------------------------------------|--------|------|------|
| | Leaf position | | | | | | | |
| | top | medial | base | mean | top | medial | base | mean |
| 0.0 (control) | 1.55 | 1.74 | 1.65 | 1.64 | 151 | 206 | 168 | 171 |
| 0.3 | 1.44 | 1.77 | 1.52 | 1.56 | 163 | 164 | 141 | 158 |
| 3.0 | 1.21 | 1.42 | 1.07 | 1.22 | 28 | 38 | 80 | 41 |
| 30.0 | 1.07 | 1.04 | 1.06 | 1.06 | 63 | 102 | 138 | 90 |
| 60.0 | 0.98 | 0.90 | 0.87 | 0.93 | 136 | 105 | 149 | 128 |

with the top ones (Tab. 3, middle column). Thus the general level of induced resistance, as calculated from lesion diameters, increased at a higher rate, and reached a higher final value in the basal leaves, than in the top leaves (Fig. 2).

The increase of the level of induced resistance proceeded simultaneously with the increase in the participation of micro-lesions in the total numbers of secondary lesions on the investigated leaves. (Fig. 3). Percentages of those lesions, primarily higher on the upper leaves (control plants), became gradually more or less equalized at all leaf positions, attaining at the highest level of induced resistance the value of ca. 50%. This means, that in comparison with control plants, the relative numbers of micro-lesions increased at the highest rate in basal leaves (Fig. 4).

Primary infection with TMV induced also considerable changes in the distribution of necrotic lesion within the challenge inoculated half-leaves. The increasing level of induced resistance resulted in a parallel increase in the relative lesion numbers in the distal zone of all leaves at the expense of their quantity in proximal zones (Fig. 5). This shift in susceptibility to infection within the leaf-blade, proceeding from the main vein of the leaf towards its periphery, was most conspicuous on the top leaves, which, as was shown before (Tab. 2), both in control series, and at a comparatively low concentration of the resistance inducing inoculum — were more susceptible to challenge infection in their proximal zones.

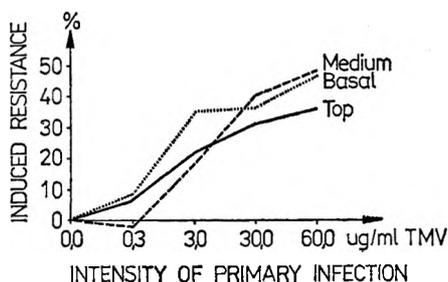
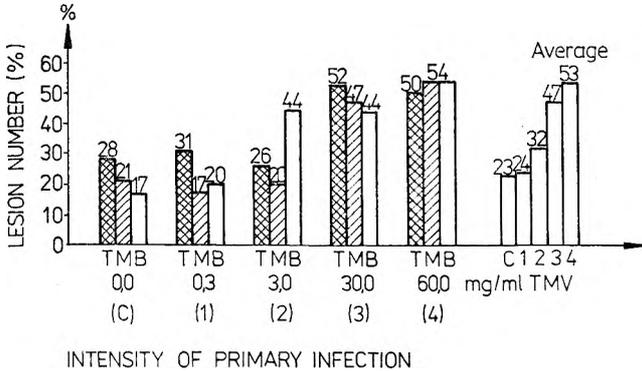


Fig. 2. Effect of intensity of primary TMV infection on the level of induced resistance in leaves of different age.



T - top leaf, M - medium leaf, B - basal leaf, C - control.

Fig. 3. Effect of intensity of primary TMV infection on numbers of secondary micro-lesions expressed as percentages of total lesion numbers at each leaf position.

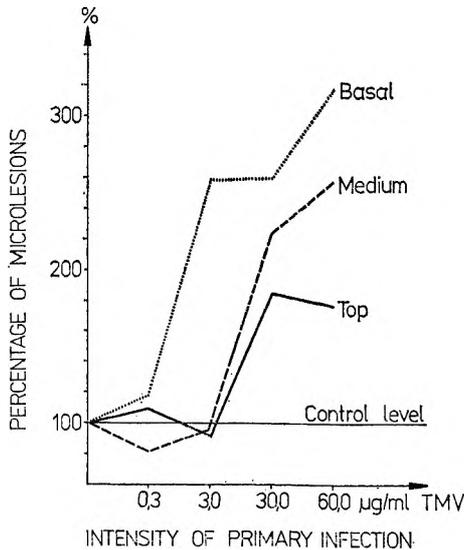


Fig. 4. Effect of intensity of primary TMV infection on the percentage of secondary micro-lesions ($\varnothing \leq 0.5$ mm) in the total lesion number at each leaf position. (Expressed as percentage of the control leaves.)

Average lesion diameters on challenge inoculated half-leaves decreased gradually in all leaf-zones investigated, parallelly with the increasing level of induced resistance, this decrease proceeded however more quickly in the proximal zones of all leaves, than in their marginal zones. It resulted thus in an increasing difference between the lesion sizes at both compared

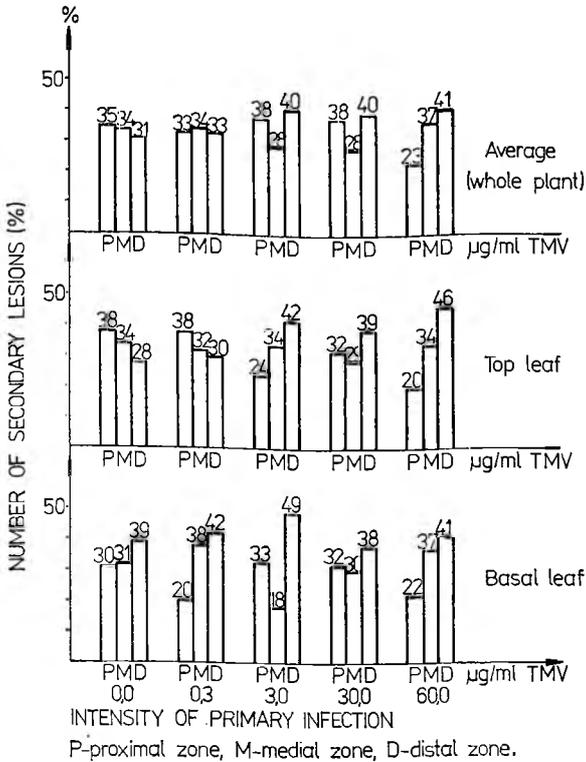


Fig. 5. Effect of intensity of primary infection on the distribution of necrotic lesions within challenge inoculated half-leaves. (Expressed as percentages of lesion totals on the leaf.)

leaf-zones, the effect of a higher level of resistance induced in the proximal zones of all leaves (Fig. 6 and 7).

The increased susceptibility to TMV infection in the distal zone of leaves with induced resistance, resulted in a simultaneous increase of the total necrotic area in that part of the leaf-blade (Fig. 8). A gradual increase of the difference in necrotised areas between the distal and proximal zones occurred in leaves at two extreme positions on the stem, parallelly to the increasing level of induced resistance. Considerable changes in this respect were again visible in the upper leaves, which in the control series were necrotised more or less uniformly in all zones, whereas only slight changes occurred in the basal leaves. Similarly as in the whole leaf, percentages of micro-lesions in each leaf zone increased parallelly to the increasing level of induced resistance (Fig. 9). In the upper leaf this increase proceeded at almost the same rate in each zone, whereas in the basal leaves, it was distinctly more intense in the proximal zone. This resulted in an increase in the difference of micro-lesion percentage between both mentioned zones in the basal leaves, as well as in a similar increase in the difference between percentages of those lesions in the proximal zones at both leaf positions.

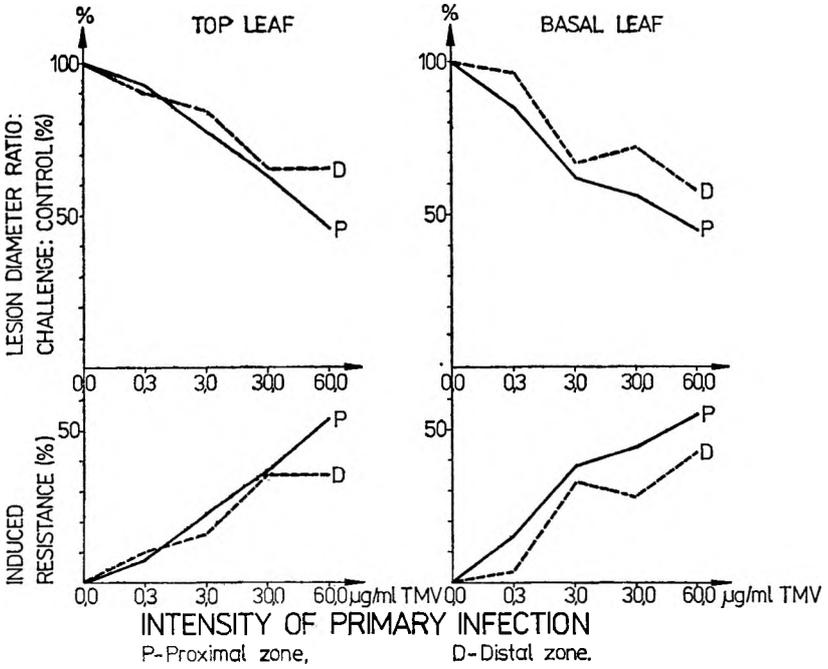


Fig. 6. Effect of intensity of primary TMV infection on lesion size reduction and the level of induced resistance in proximal and distal zones of challenge infected leaves.

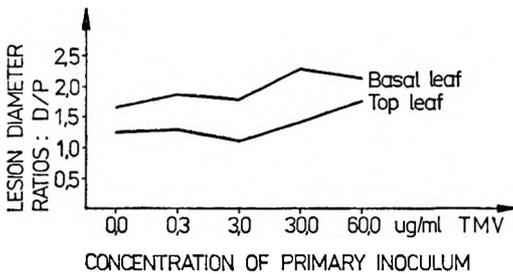


Fig. 7. Effect of increasing concentration of primary TMV inoculum on the difference between lesion sizes in the distal (D) and proximal (P) zones of challenge inoculated leaves of Samsun NN.

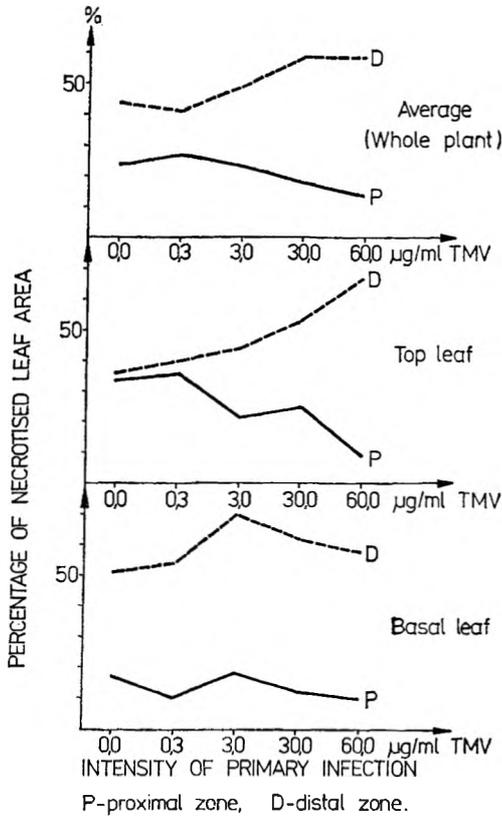


Fig. 8. Effect of intensity of primary TMV infection on the distribution of necrotised leaf tissues within challenge infected half-leaves. (Expressed as percentage of the total necrotised area.)

Discussion

Summarizing the results of the experiments described we can make the following conclusions (Tab. 4).

Leaves of Samsun NN show a natural decline in resistance to TMV, directed from the centre of the leaf towards its margins. This decline, as estimated by lesion sizes, or by necrotised areas in the distal and proximal zones of the leaf-blade, is usually greater in older leaves, than in the young ones. The increase in the average lesion size in the marginal leaf-zone is concurrent to the decrease in the relative numbers of micro-lesions in that zone, this decrease being again larger in older leaves. As the apical and central parts of tobacco leaf are physiologically older, than its marginal area, it can be supposed, that the differences observed in TMV resistance between particular zones reflect the natural differentiation of their physiological age.

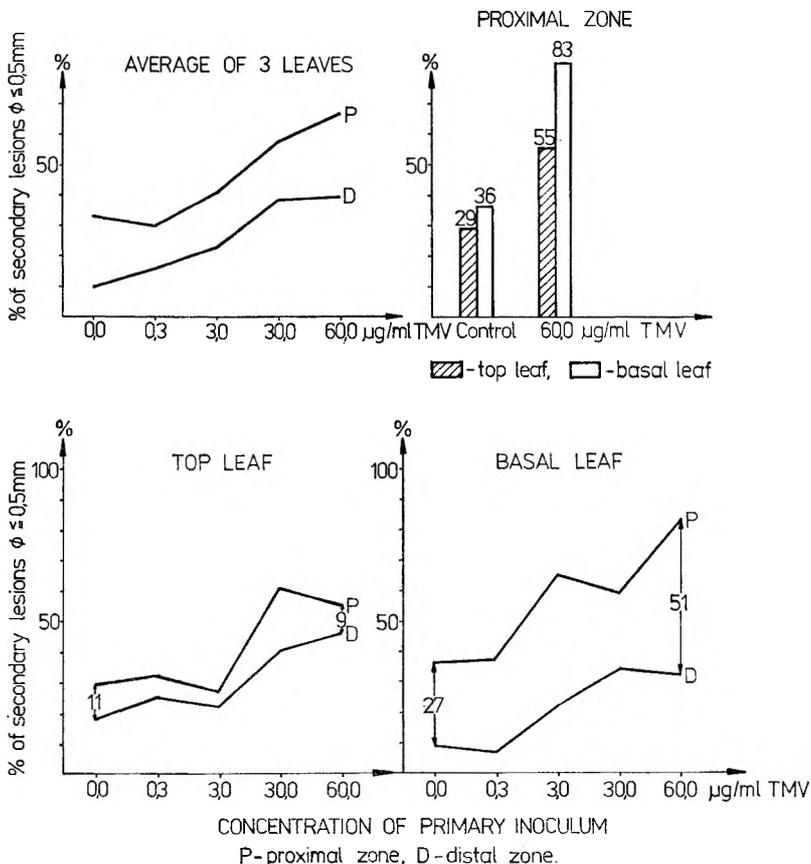


Fig. 9. Effect of intensity of primary TMV infection on the percentage of secondary micro-lesions in total lesion numbers in proximal and distal leaf zones.

Infection with TMV of the one half of the leaf increases this natural gradient of resistance in the other half even more. It becomes "steeper", which is reflected by a higher degree of acquired resistance in the proximal zone of all leaves, compared with the distal one (Fig. 5). This means, that lesion sizes in the proximal zones of all leaves were reduced to a higher degree, than in the comparable distal zones. Stimulation of resistance also induced an increase in the ratios between the distal and proximal zones in the total necrotic areas, lesion densities, and the percentage of microlesions, all those changes (shifts) being much bigger in the upper leaves, than in the basal ones. It is evident, that stimulation of resistance, evoked by primary TMV infection, was stronger in the area proximal to the main vein of the leaf, than at its margins. It also produced smaller changes, regarding lesion size and distribution, in older leaves

Table 4. Comparison of various characters of anti-TMV resistance in control (uninfected), and infected with 60 µg/ml TMV (resistant) leaves of Samsun NN.

| Ratios between »resistant« and control leaves | | | | | | |
|-----------------------------------------------|---------------|-------|---------|--|--|--|
| Character | Leaf position | | Average | | | |
| | Top | Basal | | | | |
| Lesion density | 0.90 | 0.88 | 0.75 | | | |
| % of micro-lesions 1). | 1.78 | 3.12 | 2.30 | | | |
| Necrotic area/100 sq. cm | 0.42 | 0.28 | 0.27 | | | |
| Lesion diameter | 0.63 | 0.53 | 0.57 | | | |
| % induced resistance (1. diam.) | 37 | 47 | 43 | | | |
| % induced resistance (necr. area) | 58 | 72 | 73 | | | |

| Ratios and their differences between distal and proximal leaf zones | | | | | | |
|---------------------------------------------------------------------|---------------|---------|-------|------------|---------|-------|
| Charakter | Leaf position | | | | | |
| | Top leaf | | | Basal leaf | | |
| | Control | Regist. | Diff. | Control | Regist. | Diff. |
| Lesion density | 0.74 | 2.30 | 1.56 | 1.30 | 1.86 | 0.56 |
| % of micro-lesions 1) | 0.62 | 0.84 | 0.22 | 0.25 | 0.38 | 0.13 |
| Necrotic area | 1.06 | 7.33 | 6.27 | 3.00 | 5.80 | 2.80 |
| Lesion diameter | 1.24 | 1.72 | 0.48 | 1.66 | 2.15 | 0.49 |
| % induced resist. 2) | — | 18 | | — | 13 | |

- 1). Expressed as percentage of lesion totals in each zone
- 2). Expressed as difference between proximal and distal zones

which already had a higher degree of initial resistance. These differences in the ability of different leaf zones to develop acquired resistance could reflect not only their variability in anatomic-physiological structure but could also result from the direct vicinity of the proximal zone with tissues conveying the resistance stimulus (Bozarth and Ross 1964).

Summary

Mechanical inoculation of leaves of Samsun NN tobacco with a TMV suspension causes the formation of necrotic lesions, the number and size of which depends on the physiological age of infected tissues. Lesions at the periphery of the leaf blade are usually much larger than those which are formed closer to the main vein. This difference reflects a natural decrease in TMV resistance, directed from the centre of the leaf to its margins. TMV infection of the one half of the leaf induces a certain level of resistance against viral invasion in the other half. This induction is much stronger in the area situated in direct vicinity of the main vein of the leaf, than in its marginal parts. It is also more pronounced in the young leaves as compared with the older ones. As a consequence, a gradual shift in the repartition of the necrotized tissues occurs, proceeding from the

centre of the leaf-blade to its periphery, parallelly to the increasing level of induced resistance. Reinfection of "resistant" half-leaves with TMV results in the formation of a great number of minute necrotic lesions. This decreases the calculated values of average lesion sizes, and causes their strongly skew distribution around the mean.

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SAŽETAK

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

II. TOPOGRAFIJA OTPORNOSTI PREMA VIRUSU MOZAIKA DUHANA U LISTOVIMA SAMSUNA NN

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Ako se mehanički inokuliraju listovi duhana samsuna NN suspenzijom virusa mozaika duhana, nastaju nekrotične lezije. Broj lezija ovisi o fiziološkoj starosti inficiranih tkiva. Lezije na rubu plojke obično su veće od lezija koje nastaju bliže glavne žile lista. Ta razlika ukazuje na prirodno snižavanje otpornosti prema virusu mozaika duhana koje se pruža od centra lista prema rubovima.

Infekcija jedne strane lista virusom mozaika duhana inducira u drugoj polovici lista otpornost prema invaziji virusa. Ta je indukcija mnogo intenzivnija na području koje se nalazi u neposrednoj blizini glavne žile lista nego na rubu lista. Ona je također jače izražena u mladim nego u starim listovima. Kao posljedica toga povećava se količina nekrotiziranog tkiva od centra prema periferiji plojke.

Prilikom reinfekcije »otpornih« polovina lista dolazi do formiranja velikog broja sitnih nekrotičnih lezija. To smanjuje znatno prosječnu veličinu lezija.

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