Investigation about the Necrosis Formation "Sharilka" (Aladja) on the Leaves of Field-Grown Tobacco Plants*

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I. About the Causes of the Phenomenon

The "sharilka" (aladja) is a phenomenon which has been observed on tobacco by many investigators for a long time (2, 3, 7, 11, 12). This is a specific kind of necrosis formation on the front side of the leaf blade, and most often is expressed in the form of dots, dark green in colour, which turn into dark brown as the process advances. Occasionally, the dots blend together into irregular spots, which cover a considerable part of the leaf. The different forms of "sharilka" have been described by *Grancarov* (7).

The "sharilka" manifests itself in two different cases: a) during the process of tobacco curing and yellowing, and b) on the leaves of field-grown tobacco plants. In the first case "sharilka" formation is observed when the curing and yellowing are carried out in moist, unventilated rooms, i. e. under conditions which prevent the water from being rhythmically released from the leaf. The phenomenon is causing great economic damage and is the object of many investigations, in which it is not only described (7), but also the conditions necessary for its manifestation are examined and ways and means of averting its appearance are looked for (11). Of special interest in clarifying the nature of "sharilka" occurring during the process of curing tobacco leaves are the studies carried out by *Bailov* and *Andrejceva* (3, 12), as they proved experimentally that this phenomenon is associated with the accumulation of chlorogenic acid in the leaves.

Furthermore, the "sharilka" is observed also on tobacco plants grown in the field (2). "Sharilka"-affected leaf is shown in Fig. 1. This kind of "sharilka" could be regarded as identical with the so-called "verderame" which was reported by the Italian authors *Gigante* (14) and *Marcelli* (16), but it seems that it differs from the "bassara" which has been observed on tobacco plants in Yugoslavia (5, 6, 9, 10). During the humid summers of 1967 and 1968, the tobacco grown in South-West Bulgaria suffered very much from "sharilka" and this made it necessary to carry out more detailed studies of this phenomenon.

There are, however, but few studies of "sharilka" on plants grown in the field.

In the present work we have aimed at elucidating the causes which lead to the appearance of this phenomenon.

According to *Yankulova* (unpublished data) the "sharilka", which appears on field-grown tobacco plants, is not caused by pathogenic organisms. The phenomenon is most probably of a physiological nature. Of special importance in favour of this concept are the following considerations, which we used as the starting point in our studies:

1. "Sharilka" is mainly observed on Oriental tobaccos, which are distinguished by the thin structure of their leaves. On tobaccos with thicker leaf structure, such as Virginia and Burley types, it is only rarely observed — during periods of very favorable conditions for its appearance.
2. It manifests itself only during periods of abundant and frequent rainfalls, mostly during the late summer months, i. e. during the period in which tobacco leaves begin to ripen.
3. It mainly affects the low standing leaves, which are also the thinnest, and in the stage of overripening.
4. Especially susceptible to "sharilka" are the leaves of tobaccos cultivated on poor soils.

In short, it seems that two factors are of prime importance for the appearance of "sharilka": leaf structure and ripeness and high moisture. In fact, "sharilka" manifests itself when these factors are in a favorable combination, i. e. when the tobacco plant, during the period when the leaves begin to overripen, finds itself under conditions of water excess. In essence, the overripening is a process of senescence, which is characterized by cell dehydration, slowly operating synthetic processes (13), breaking down of the polymeric substances, aging of colloids, degeneration of cell struc-

Figure 1. "Sharilka"-affected leaf of the variety *Nevrokop 5*.

* Presented at the 5th International Tobacco Scientific Congress, Hamburg, September 1970.

tures and disorders in the properties of cell membranes (19). It is only logical to assume that the senescing leaf has a decreased water binding capacity and cannot structurize a large quantity of water. The presence of unbound water in such a leaf, in quantities higher than normal, probably acts as a kind of stress which causes disorders in the labile organization of the senescing cell, their final expression being necrosis of the „sharilka“ type.

In order to prove experimentally our assumption about the importance of the water binding capacity as a factor for the appearance of „sharilka“ on tobacco leaves, we tried to determine the quantity of the main cell colloids — pectins and proteins. These substances have a direct relation to the processes of water retention and water binding, as they possess polar groups and, as a result of this, can hydrate and immobilize part of the water in the cell.

We have studied also the ratio of free and bound water, as the quantity of the latter is an indicator of the water binding capacity of the protoplasm. Besides, it is a well-known fact that the quantity of free water in plants during the various stages of development is different, being considerably higher at the end of the processes of formation of flower parts (1).

MATERIAL AND METHODS

In the experimental work we used objects which were susceptible and non-susceptible to „sharilka“, namely:

a) Leaves of lower positions of the „sharilka“-susceptible Oriental tobacco varieties, Harmanliiska basma 163, Nevrokop 5 and Djebel 359, and of the „sharilka“-resistant tobacco varieties, Burley 21, S-21 and S-46, which are of the Burley type, and Virginia I, VSP-26 and VSP-23, which are of the Virginia type.

b) Leaves from low, middle and upper positions of the above-mentioned three Oriental tobacco varieties, the susceptibility to „sharilka“ of which decreases considerably from the low to the upper leaf positions.

The experimental plants were cultivated under the conditions prevailing in 1968 in the Experimental Base, Vrana. The samples for investigation were taken after heavy rain, i. e. at a moment preceding the appearance of „sharilka“. The plants from which leaves were taken for investigation were marked so that it could be seen whether, one or two days after the rain, „sharilka“ had appeared in the same positions. In all cases of Oriental tobacco varieties, „sharilka“ was observed on the lower leaves and only rarely on the middle leaves. The leaves of Virginia and Burley tobacco varieties remained unaffected or were affected but little by „sharilka“.

For the sake of brevity, the Virginia and Burley type tobaccos will be called „sharilka“-resistant, but this term does not have an absolute meaning, as under certain extreme conditions these tobaccos could also suffer from „sharilka“, though to a considerably smaller extent than the Oriental tobaccos.

Total, free and bound water was determined by the *Marincik* method (8). A 70 per cent saccharose solution was used as the water absorbing medium. After a preliminary testing of several saccharose concentrations, it was established that such a concentration was most suitable.

Pectins were determined as isolated pectins by the *Pyriki* and *Moldenhauer* method (17).

Proteins were determined by precipitation with a 5 per cent solution of trichloroacetic acid. The sediment was mineralized by *Kjeldahl's* method by application of about 1 g selenic catalyst, composed of: 100 g — K_2SO_4 , 10 g — $CuSO_4 \cdot 5 H_2O$ und 2 g Se. The ammonia evolved during the following distillation was bound by a 4 per cent solution of H_3BO_3 using a mixed indicator, consisting of 1 per cent methyl red solution and 0.2 per cent solution of bromo-cresol green in 80 per cent alcohol, in a ratio of 1:5.

The experimental data were subject to mathematical analysis using the function $\Psi^{2/n, t/*}$ and were significant.

RESULTS

The experimental data show that the protein content decreases from top to bottom (Table 1). This „gradient“ is best demonstrated by the variety Harmanliiska basma 163, the upper leaves of which contain 10 per cent more proteins than the lower leaves. The difference in the protein content of the upper and the low leaves for the other varieties is no less than 8 per cent. The susceptibility to „sharilka“ consequently is maximal in the low leaves and corresponds to the lowest protein content, and, conversely, the least susceptible to „sharilka“ upper leaves are the richest in proteins.

It was also established that the low leaves, which are mainly susceptible to „sharilka“, contain fewer pectins than the middle and upper leaves (Table 1). For example, in the variety Harmanliiska basma 163 this difference amounts to 4.17 per cent pectins in the low leaves as against 5.98 per cent and 5.59 per cent respectively for the leaves at the middle and the upper part of the plant.

These results were confirmed by the data obtained when determining the pectin content of the less susceptible to „sharilka“ Virginia and Burley type tobacco varieties, and also of the very susceptible Oriental tobacco varieties. The Virginia and Burley type tobacco varieties are distinguished for their higher content of pectin (of the order 5.6–6.0 per cent) than the Oriental tobacco varieties, which contain some 4.0–4.5 per cent (Table 2).

The studies of the ratio between bound and free water in tobacco leaves (Table 3) indicated that for the Virginia and Burley type tobacco varieties, which are resistant to „sharilka“, this ratio is in favour of the bound form. The latter amounts to some 65–78 per cent of the total water content of these varieties, which is indicative of a high water binding capacity.

* Barov, K., V. Jovceva: *Rastenievadni Nauki*, 1964, 1, No. 7, 51–63.

Table 1. Pectin and protein content of leaves from lower, middle and upper positions of "sharilka"-susceptible Oriental tobacco plants.

Oriental varieties	Leaf position	Pectins (as isolated pectins)		Protein	
		% fresh weight	% dry weight	% fresh weight	% dry weight
Djebel 359	lower	0.65	3.94	1.30	7.90
	middle	1.14	5.24	3.13	14.41
	upper	0.95	4.88	3.29	16.84
Harmanliiska basma 163	lower	0.69	4.17	1.47	8.86
	middle	1.20	5.98	2.91	14.47
	upper	1.00	5.59	3.24	18.12
Nevrokop 5	lower	0.49	3.65	1.47	11.04
	middle	0.88	4.80	2.64	14.37
	upper	0.84	4.45	3.67	19.33

An opposite ratio was established for the "sharilka"-susceptible Oriental tobacco varieties, in which the share of bound water amounted to 31–40 per cent, while the share of the free form was much greater —

Table 2. Pectin content of "sharilka"-resistant Burley and Virginia types and "sharilka"-susceptible Oriental tobaccos (leaves from lower positions).

Type	Variety	Pectins (as isolated pectins)	
		%/fresh weight	%/dry weight
Burley	Burley 21	1.59	6.08
	Burley S-21	1.61	6.00
	Burley S-46	1.64	5.90
Virginia	Virginia I	1.48	6.70
	VSP-26	1.74	6.36
	VSP-23	1.64	5.50
Oriental	Harmanliiska basma 163	0.80	4.17
	Nevrokop 5	0.88	4.11
	Djebel 359	0.94	4.50

in the order of 60–69 per cent. Similar results were obtained by studying leaves from different positions on the stem. Highest percentage of bound water was established in the upper leaves, and lowest — in the low ones (Table 4). And, furthermore, the difference is not so great between the leaves of the middle and the upper parts — about 3–4 per cent, while between the lowest and the upper leaves this difference is considerable. For example, for the variety Djebel 359 the low leaves contain some 21.67 per cent bound water as against 71.72 per cent for the upper leaves, the difference being over 50 per cent. The trend is the reverse as regards their free water content, the low leaves contain considerably more free water, in the order of 45–61 per cent, and the upper leaves considerably less — 9–17 per cent.

These differences between the low and the upper leaves, in fact, reflect their water binding capacity. This is much smaller in the lower, susceptible to "sharilka" leaves, and this is the cause of the higher quantity of free water in them than in the upper "sharilka"-resistant leaves, which possess great water binding capacity and contain less free water.

Table 3. Free water and bound water content of "sharilka"-resistant Burley and Virginia types and "sharilka"-susceptible Oriental tobaccos (leaves from lower positions).

Type	Variety	Total water %	Free water %	Bound water %	Free water % of total water	Bound water % of total water
Burley	Burley S-21	73.50	23.35	50.15	31.76	68.24
	Burley S-46	72.78	26.39	46.39	36.25	63.75
Virginia	Virginia I	70.25	22.84	47.51	32.51	67.49
	VSP-26	72.65	25.03	47.62	34.45	65.55
	VSP-23	70.17	19.65	51.11	28.00	78.00
Oriental	Harmanliiska basma 163	80.86	51.65	29.21	63.87	36.13
	Nevrokop 5	81.36	48.91	32.44	60.11	39.89
	Djebel 359	84.61	58.41	26.19	69.03	30.97

Table 4. Free water and bound water content of leaves from lower, middle and upper positions of „sharilka“-susceptible Oriental tobacco plants.

Oriental varieties	Leaf position	Total water %	Free water %	Bound water %	Free water % of total water	Bound water % of total water
Djebel 359	lower	83.56	61.89	21.67	74.07	25.93
	middle	78.28	10.90	67.38	13.92	86.08
	upper	80.50	8.77	71.73	10.89	89.11
Harmanliiska basma 163	lower	83.42	45.03	38.39	53.97	46.03
	middle	79.89	17.68	62.21	22.13	77.87
	upper	82.22	17.00	65.22	20.67	79.33
Nevrokop 5	lower	86.69	49.30	37.39	56.80	43.20
	middle	81.64	17.09	64.55	20.93	79.07
	upper	81.02	13.88	67.14	17.13	82.87

To the factors which contribute to the lower content of free water, or to the resistance to “sharilka” of the upper leaves, should also be added a known peculiarity of these leaves: they have greater number of stomata per unit of area and transpire more intensively than do the lower leaves (4). It could be accepted that the upper leaves do not suffer from “sharilka” because they are able to cope more efficiently with the excess water than do the low leaves: on the one hand, as was indicated, they possess a greater water binding capacity and consequently, could bind a greater quantity of the water received; on the other hand, they were able to rid themselves of the excess water as they transpire more intensively, so that in the end, water in free form does not accumulate in their cells.

In consideration of these results, it is logical to assume that factors of different character, in the first place insufficient nutrition, which leads to a lowering of the water binding capacity of protoplasm, and, further, to a greater quantity of free water, could, to some extent, contribute to the appearance of “sharilka” on tobacco. In this aspect could be explained the fact that leaves from tobacco plants grown on poor soils are very susceptible to “sharilka”.

The appearance of “sharilka” in the field, consequently, could be kept under control by an appropriate regime of nutrition, which should give the plants the possibility of synthesizing colloid substances in such form and quantities as are needed for a higher water binding

capacity. In consideration of this, an experiment was carried out, aimed at studying tobacco “sharilka” under different regimes of fertilization. A fertilization experiment was carried out using the scheme provided by *Kitipov* (Table 5). As is seen from the data, the plants suffering from “sharilka” decreased in number after nitrogen fertilization. The variant P₄ shows the lowest percentage of “sharilka”-affected plants — 50 per cent, i. e. almost twice less than the unfertilized control, and this proves the positive role of phosphorus in decreasing “sharilka” susceptibility.

Of course, these results are of importance merely as a beginning of a new trend in the control of “sharilka” and need to be supplemented by further, more detailed investigations.

DISCUSSION

The results so far obtained indicate that “sharilka” is a physiological phenomenon observed on overripening leaves under conditions of water excess and is linked above all to the decreased water binding capacity of protoplasm. It has been established that Virginia and Burley type tobaccos, which have thicker leaves, and also the upper leaves of Oriental tobaccos, possess greater water binding capacity and seldom suffer from “sharilka”. The senescing, overripe low leaves of Oriental tobacco varieties are characterized by a low water binding capacity and are very susceptible to “sharilka”.

The water binding capacity is a summary indicator for the quantity and state of cell colloids, mainly of pectins and proteins. The studies indicated that a positive correlation exists between the quantity of pectins and proteins in the cells and the resistance to “sharilka”. It should be added, however, that in addition to the above-mentioned substances, other polymers, for example, the complexes of ferulic acid with certain glycoproteids, also take part in the processes of water binding (15).

The leaves which do not suffer from “sharilka” possess a greater water binding capacity and are able to bind a considerable part of the water which the plant

Table 5. Effect of fertilization on “sharilka” formation on leaves of the Oriental variety Nevrokop 5.

Fertilizers applied	Total number of plants	Number of “sharilka”-affected plants	% “sharilka”-affected plants
1. Control-not Fertilized plants	139	128	92.08
2. N	138	102	73.91
3. N + PK	134	104	77.61
4. N + P ₂ K	119	92	77.31
5. N + P ₄ K	147	99	67.34
6. N + P ₄	128	64	50.00

receives during the periods of abundant and frequent rain. The "sharilka"-susceptible leaves have a lower water binding capacity. For that reason, the water in such leaves could not be bound to a sufficient degree and the greater part of it accumulates in the cells in free form. According to *Shaw and Manocha* (18), some days after detached wheat leaves are put in water, in some cells disorders in their fine structure were observed. In this case the increased rate of hydration caused a considerable increase in volume of the cytoplasm, which led to contraction and fragmentation of vacuoles, breaking down of tonoplasts and loss of the cellular integrity. In this connection, it could be supposed that the higher water content in senescing, overripe tobacco leaves, namely, is the cause of the degenerative changes in the cells. The latter find visible expression in the specific necrosis of small sections of leaf tissue, which take the form of "sharilka".

The nature of the biochemical processes which lie in the base of "sharilka" formation, and also the cyto-anatomical changes of the "sharilka"-damaged leaf are discussed in the following paper.

CONCLUSIONS

1. "Sharilka" is a physiological disturbance of tobacco plants of Oriental type.
2. It is caused by water stress, and, more specifically, by water excess during the period when the leaves begin to overripen.
3. Susceptibility to "sharilka" is associated with a decreased content of pectins and proteins.
4. The latter fact predetermines a lower water binding capacity, or a higher content of free water in the tissues suffering from "sharilka".

SUMMARY

A specific dot-like necrosis formation on Oriental type tobacco, called "sharilka" was studied. The phenomenon is of a physiological nature. It manifests itself under conditions of water excess during the time when the leaves begin to become overripe. It was established that susceptibility to "sharilka" is connected with a low content of pectins and proteins. The latter fact predetermines the lower water binding capacity of protoplasm or higher content of free water in the tissues which suffer from "sharilka".

The raised quantity of free water as a possible source of degenerative cell changes, which become apparent on the leaf in the form of "sharilka", was considered.

ZUSAMMENFASSUNG

Bei „sharilka“, einer spezifischen fleckenförmigen Nekrose der Blätter von Orienttabaken, handelt es sich um ein physiologisches Phänomen, das bei einem Überangebot an Wasser in der Zeit auftritt, wenn die Blätter anfangen, überreif zu werden. Die Anfälligkeit für „sharilka“ nimmt zu, wenn die Pflanzen einen geringen

Gehalt an Pektinen und Proteinen aufweisen. Der Mangel an Proteinen führt zu einer geringeren Wasserbindung durch das Protoplasma, das freie Wasser nimmt im Gewebe zu, und es kommt zur Ausbildung von „sharilka“.

Diese Zunahme freien Gewebewassers wird als mögliche Ursache degenerativer Zellveränderungen aufgefaßt, die im Blatt als „sharilka“ in Erscheinung treten.

RESUME

Des recherches ont été faites concernant une formation nécrotique sous forme de points, appelée »sharilka«, et observée sur un type Oriental de tabac. C'est un phénomène d'ordre physiologique. Celui-ci se manifeste par une hydratation excessive lorsque les feuilles commencent à être trop mûres. On a aussi établi que la sensibilité des feuilles de tabac pour le »sharilka« dépend d'une faible concentration en pectines et protéines. Il en découle une capacité amoindrie de fixation d'eau par le protoplasme et donc une concentration supérieure d'eau non fixée dans les tissus, qui souffrent de »sharilka«.

On a considéré cette quantité supérieure d'eau non fixée comme une cause possible de la dégénérescence des cellules, qui devient apparent sur les feuilles sous la forme de »sharilka«.

REFERENCES

1. Alekseev, A. M.: The water regime of plant cells in connection with the metabolism and the structure of cytoplasm; Timir ctenie, Moskva, 1969.
2. Atanasov, D.: Research works of the Higher Institute of Agriculture "Vasil Kolarov", Plovdiv, 1954, vol. II, p. 261.
3. Bailov, D., Stefanova, M., Andrejceva, M.: About the nature and the causes of tobacco flecks (aladza); Comptes rendus BAN, 1957, vol. X, No. 6, p. 509-512.
4. Rubin, B. A.: Handbook of plant physiology; V. Shkola, Moskva, 1961.
5. Beaka, Hr.: Contribution to the study of tobacco bassara; Tutun, 1963, No. 3-4, p. 66-68.
6. Bucovac, P.: Contribution to the study of tobacco bassara; Trudovi na institutot za tutun, Prilep, 2, No. 2, 133-163, 1954.
7. Grancarov, H.: Sharilka (aladza) of cured tobacco; Tjutjunov Pregled, 1936, V, No. 8-9.
8. Marincik, A. F.: Peculiarities of the physiological processes in connection with the state of water in the leaves and the productivity of sugar beet; Collection: Biologiceskie osnovi oroshaemogo zemledelja, published by the Academy of Sciences of the USSR, Moskva, 1957, p. 584-595.
9. Mickovski, J.: Effect of different factors upon the appearance of bassara on tobacco; Tutun, 1962, No. 10-11, p. 261-311.
10. Todorovski, P.: Tobacco bassara on leaves picked at different stages of ripeness; Tutun, 1963, No. 8, p. 161-170.