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BACTERIA IN THE XYLEM OF SUGAR-BEET LEAVES ATTACKED BY *CERCOSPORA BETICOLA* SACC.

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Introduction

Sugar-beet is cultivated on a very large area in Yugoslavia and is an important crop for the economy of the country. Like many other cultivated plants, sugar-beet is attacked by many fungal and viral diseases. Among them the most harmful is the leaf spot disease which is caused by fungus *Cercospora beticola* (Marić 1969, 1974). This fungus can be controlled by means of pesticides.

C. beticola belongs to the group of Fungi imperfecti and can multiply with conidia. If conidia enter the leaf of sugar-beet, light green spots develop after 15 days. The spots soon become larger, rounded, light brown, and reach a diameter from 2 to 3 mm. A cross section of the spot shows that the leaf is thinner in the middle of the spot. The mycelium of *Cercospora* occurs in the central part of the spot where the leaf cells are dead. The marginal part of the spot consists of living and turgescent cells whose walls are partially suberized and give a positive reaction with Sudan III.

The living marginal part of *Cercospora* spots was brown red. According to Schmidt (1929) and Noll (1956) the red colour of spots resulted from the presence of anthocyanine in the cell sap. However, recent investigations have shown that *Beta vulgaris*, other species of the family *Chenopodiaceae* and many other related families belonging to the group of *Centrospermae* instead of anthocyanine always have another pigment named betacyanine. This cell sap pigment has a special chemical structure different from anthocyanine. In betacyanine the chromophoric part of the molecule is formed by a 1,7-diazaheptamethinium-system (Reznik 1975). Sometimes, in yellow forms of *B. vulgaris* a yellow pigment appears instead of betacyanine. This pigment named betaxanthine is chemically closely related to betacyanine.

The mycelium of *C. beticola* which attacks a young sugar-beet leaf cannot enter this living and coloured part of the spot which represents a barrier for the spreading of fungus. This living part of the spot is without mycelium at least in young spots. However, in old leaves the marginal part can no longer prevent the spreading of the fungus, which then penetrates into the surrounding tissues.

As the sugar-beet is a very important crop plant for Yugoslav agriculture, we decided to investigate the leaves attacked by *Cercospora* with electron microscope.

Material and Methods

The leaves of sugar-beet for this investigation were collected in the rural community of Orašje near Brčko in Bosnia in October 1978 (Fig. 1a). They had a large number of spots caused by *C. beticola* Sacc. The identification of this fungal disease was kindly done by Ing. Devleta Buturović (Institute of Agriculture, Butmir, Sarajevo) who established the presence of characteristic conidia.

For electron microscopy small leaf pieces were fixed in buffered glutaraldehyde and afterwards in OsO_4. The samples were dehydrated in ethanol and then embedded in Epon 812. The material was subsequently cut with a diamond knife, stained with uranyl magnesium acetate (Kimura et al. 1975) and lead citrate and observed in a JEM 100 B electron microscope.

Apparently completely healthy leaf parts were taken for investigation. These leaf parts were placed among the spots and were at least two to three mm away from the spot margins. Moreover, we chose only those leaf parts which contained a vascular bundle. We wanted to establish whether the apparently healthy leaf parts were altered by the fungal infection.

Results

Consequently, the investigated leaf parts did not include the spot parts but only the leaf parts among spots, which appeared to be healthy. In spite of that, a large number of bacteria were found in the xylem during the examination of ultrathin sections (Fig. 1b, 2, 3). The bacteria were present only in the tracheids and vessels and not in the cells of xylem parenchyma. It is necessary to point out that in the vessels a quantity of organic material was present which could serve as food for bacteria. Appart from bacteria single hyphae which probably belonged to *C. beticola* were observed in the xylem (Fig. 1b, 2).

The healthy leaf parts which were more than 5 to 6 mm away from *Cercospora* spots were also examined but in these tissues bacteria and hyphae were not found. Therefore, we could conclude that bacteria and hyphae entered the tissue through the spots.

The bacteria were spherical in form or a little elongated. Their width was 400 to 500 nm and the length up to 1,200 nm. On the surface of the bacteria there was an outside part of cell wall about 10 nm thick and consisted of two darker peripheral layers and a lighter middle layer

Fig. 1. a — Sugar-beet leaves with a large number of spots caused by *Cercospora beticola*. b — Ultrathin section through the xylem near the spot. Many bacteria and the cross-section of a hypha are visible. Bar represents 500 nm.

Sl. 1. a — Listovi šećerne repe s brojnim pjegama prouzročenim od gljivice *Cercopsora beticola*. b — Ultratanki presjek kroz ksilem u blizini pjege. Vidi se veći broj bakterija i presjek kroz hifu. Skala predstavlja 500 nm.

Fig. 2. To the right, a large number of bacteria with slightly wrinkled and scaly cell walls. In the cells many electron dense grains. Above left a part of a hypha is visible. Bar represents 300 nm.

Sl. 2. Desno veći broj bakterija s ponešto nabranom i kljuskastom stijenkom. U bakterijskim stanicama veći broj zrnaca koja ne propuštaju elektrone. Lijevo gore dio jedne hife. Skala predstavlja 300 nm.

Fig. 3. In the middle a bacteria with fairly smooth cell wall. The place, where the outer part of cell wall is three layered, is marked with an arrow. Here and there the cell membrane is visible. In the marginal cell parts many ribosomes (r) and in the middle DNA filaments (f) are situated. In the lower cell part many electron dense droplets are prominent. Bar represents 200 nm.

Sl. 3. U sredini slike vidi se bakterija s prilično glatkom staničnom stijenkom. Strelica pokazuje vanjski dio stanične stijenke koji je troslojan. Na više mjesta vidi se dobro i stanična membrana. U rubnom dijelu stanice vidi se velik broj ribosoma (r) a u sredini niti DNK (f). U donjem dijelu slike zapaža se nekoliko intenzivno obojenih kapljica. Skala predstavlja 200 nm.

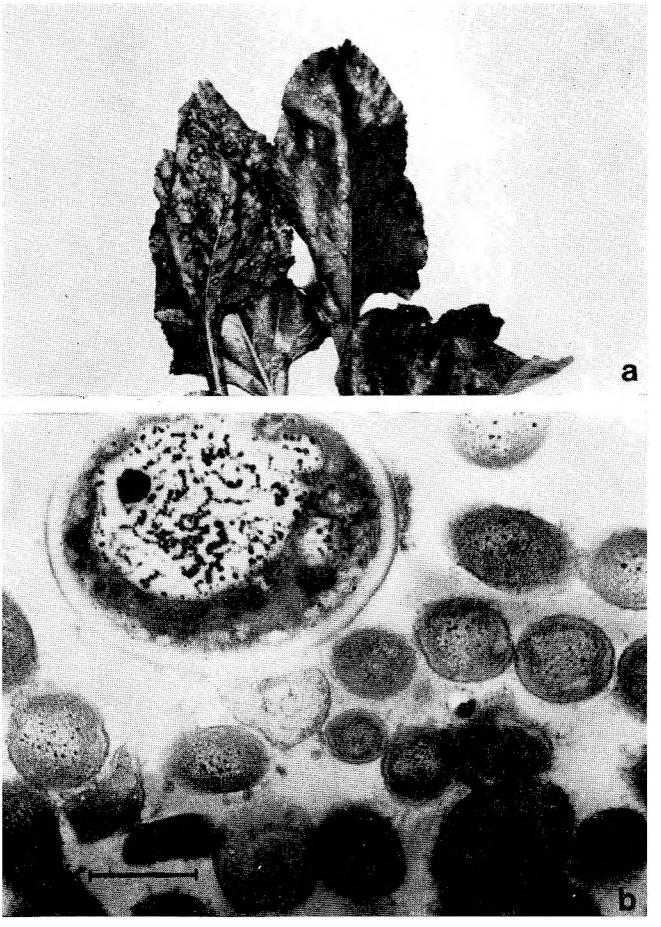

Fig. 1. — Sl. 1.

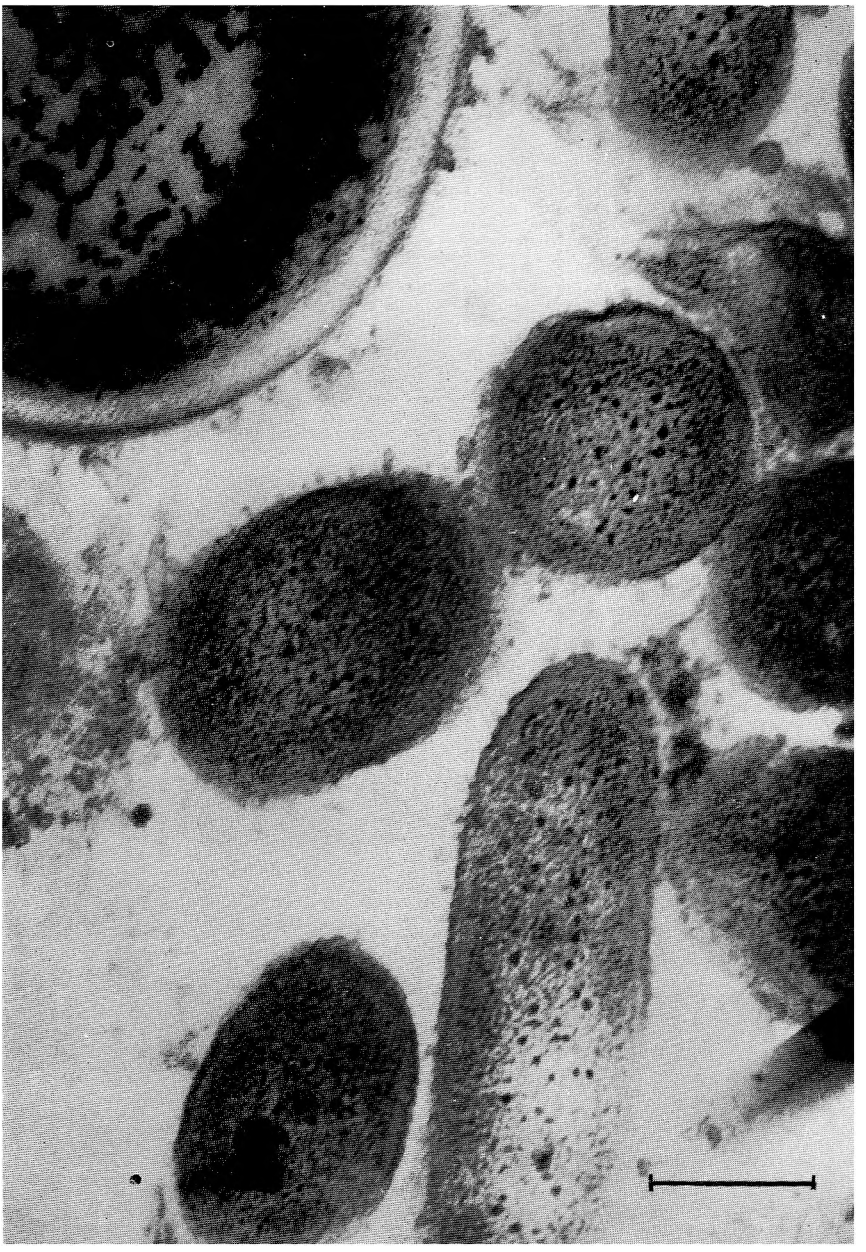

Fig. 2. — Sl. 2.

Fig. 3. — Sl. 3.

(Fig. 3; cf. Nyland et al. 1973). This wall was moderately wrinkled in some places. The wrinkling was never as pronounced as it was in bacteria which were the cause of phony peach disease (Nyland et al. 1973). In many cases we could observe that the moderately wrinkled cell walls had an irregular scaly structure (Fig. 2).

In the inside of the bacterium, the cell membrane was clearly visible (Fig. 3). The peripheral parts of protoplast contained a large number of round bodies which were intensively stainable and had a diameter of 15 nm. It seems that they represent the ribosomes. In the central cell part many intertwined and well stained filaments were situated, which probably belong to the DNA.

In the lower part of the bacterium presented in Fig. 3 a large cell part is visible in which many dark droplets are present.

The morphology of bacteria observed was a little different and it is probable that they do not belong to the same species.

In some preparations single hyphae with a diameter of 3 μm were present in the xylem. They had very thick cell walls and different contents of the cells (Fig. 1a, 2).

Discussion

In the leaf parts which were situated at a small distance from the *Cercospora* spots numerous bacteria and even hyphae were found. According to investigations of Noll (1956) the spots formed by Cercospora are surrounded by a margin which consists of living cells sometimes containing red cell sap. In the leaves of normal vitality this margin represents a barrier through which the hyphae can not penetrate the surrounding living leaf parts. However, in the old leaves the barrier is not more efficient and the hyphae can penetrate through it and spread out of the spot.

It must be stressed that this investigation was carried out on old leaves in October when the barrier was no longer resistent. Therefore, the hyphae could pass through the barrier and came to other leaf tissues. Together with the fungus, the bacteria could also spread through other tissues which formerly were without them.

That the bacteria can live in the xylem and penetrate through xylem the various plant organs causing a systemic disease, was known at the beginning of 1970s. At this time the interest in the ethiology of some insufficiently studied "virus" diseases was revived and it was established that their causes were not viruses but bacteria localized in xylem. It seems that Plavšić-Banjac and Maramorosch (1972) first discovered rickettsia-like organisms in the xylem of sugarcane which were the agents of ratoon stunting disease. Later Kao and Damann (1978) identified these organisms as bacteria from the group of *Actinomycetales*. In a similar manner Pierce's disease of grapevine was formerly considered a virus disease. Goheen et al. (1973) described in situ the bacterium which was the cause of this disease, and Mollenhauer and Hopkins (1974) studied its properties. Phony peach disease was also an important disease caused by xylem localized bacteria which was regarded in the past a virus disease (cf. Nyland et al. 1973, French, Christis and Stassi 1977).

It is specially interesting that rickettsia-like organisms were found by Nienhaus and Schmutterer (1976) also in sugar-beet. It seems that these organisms provoke the rosette disease of sugar-beet.

Summary

The fungus *Cercospora beticola* causes a large number of spots on the leaves of sugar-beet which considerably diminish the yield. In order to establish whether the apparently healthy leaf parts among the spots were altered, these leaf parts were investigated by means of electron microscope. The investigated places were from two to three nm away from the spot margins which were of a red brown colour. In the xylem of these parts numerous bacteria and some fungal hyphae were found. In the apparently healthy leaf parts which were 5 mm away from leaf spots, we could find neither bacteria nor fungi. Obviously the bacteria entered the healthy tissues through the spot but they could not spread very far from it.

This investigation was carried out in October 1978, that is at the end of the growing season of sugar-beet. It would be interesting to investigate whether bacteria appear in other stages of cultivation in order to ascertain their role on the decay of sugar-beet.

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SAŽETAK

BAKTERIJE U KSILEMU LISTOVA ŠEĆERNE REPE NAPADNUTIH GLJIVICAMA *CERCOSPORA BETICOLA* SACC.

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Gljivica *Cercospora beticola* stvara na listovima šećerne repe mnogobrojne pjege koje znatno smanjuju prirod. Da bi se ustanovilo da li se u mjesecu listopadu alteriraju prividno zdrava područja listova između pjega, istražena su elektronskim mikroskopom ta područja. Ta su mjesta na listovima bila 2 do 3 mm udaljena od ruba pjega koji je bio crvenosmeđe obojen.

Istraživanja su pokazala da se u tim područjima nalaze samo u ksilemu mnogobrojne bakterije i poneke hife vjerojatno od vrste *C. beticola*. U zdravim područjima listova, koji su bili još više udaljeni od pjega, nismo mogli pronaći ni gljivinih hifa ni bakterija. Na osnovi tih zapažanja može se zaključiti da je sigurno da bakterije ulaze u prividno zdrava tkiva lista kroz pjegu, ali se ipak ne mogu u znatnijoj mjeri udaljiti od područja pjege.

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