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Investigations into the cause of the phloem necrosis disease of Coffea liberica in Surinam, South America

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Abstract

The presence of the flagellate *Phytomonas leptovasorum* Stahel in the phloem vessels of *Coffea liberica* Bull. ex Hiern., suffering from phloem necrosis, was once more demonstrated. The association of the organism with the multiple division of sieve tubes in affected trees was confirmed. The different nature of a wilting disease caused by the fungus *Ceratocystis fimbriata* Ellis et Halst. has again been established.
No viruses, bacteria, and nematodes could be detected as pathogens of phloem necrosis.
Although phloem necrosis is found almost exclusively in mature trees, it was possible to infect young ones by root grafting; the symptoms were, however, less acute here than in older trees. Other species of *Coffea* can be affected, either by natural infection or by artificial inoculation, but there too, the symptoms – internal as well as external – are less severe than in mature trees of *C. liberica*. The way of natural infection is still unknown. Hemipteran insects are suspected to transmit the disease, since such insects are known to be vectors of *Phytomonas* (= *Leptomonas*) *davidi* Lafont in *Euphorbia* spp. Attempts to grow the organism in pure culture have failed and hence its pathogenicity could not be established. However, the absence of any evidence towards fungi, viruses, nematodes or bacteria supports the hypothesis that *Phytomonas leptovasorum* Stahel is the causal agent of the phloem necrosis disease of *Coffea liberica* in Surinam.

Introduction

The losses due to the phloem necrosis disease of *Coffea liberica* in Surinam did not attract worldwide attention, as the disease occurred only in a small, relatively unimportant coffee growing area and furthermore in a coffee species of limited economic importance.
Stahel's[2] findings (1931, 1932, 1933) that organisms of a protozoan nature were closely

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[2] This paper is dedicated to Prof. Dr. G. Stahel, whose meticulous observations proved to be correct after 30 years.

associated with the disease, caused considerable scientific interest at that time, this being the first recorded instance of these organisms as pathogens in non-laticiferous plants.
The 2nd World War, however, prevented further research on this subject. After the war different views were put forward, when a fungus could be isolated from coffee trees showing disease symptoms resembling those of phloem necrosis. The resulting controversy led to more investigations by van Emden and van Suchtelen (1959) and van Emden (1962), who stated that the disease symptoms in the field could be attributed either to *Ceratocystis fimbriata* Ellis et Halst. (= *Ophiostoma* (*Rostrella*) *coffeae* (Zimm.) v. Arx) or to the organism described by Stahel.
At that stage it was considered opportune to make a renewed study of the phloem necrosis disease, its relation to phytoflagellates and other plant pathogens. In 1960 research on the disease was started afresh and in 1962 and 1963 the present author reported his findings in interim papers. A detailed account of the work, which was terminated in 1963, is presented in this paper.

Survey of literature

In the beginning of this century an unknown disease was reported in the *C. liberica* plantations in Surinam (van Hall, 1906); the symptoms were a wilting and rapid death of mature trees; consequently it was called "wilting disease" and attributed to fungal root rot. Kuyper (1913) was the first to do more research on the cause, but his anatomical investigations did not reveal any aberrations in the tissue of affected coffee trees. He suggested that adverse growing conditions caused the symptoms. Stahel (1917), however, found necrosis and gummosis of sieve tubes in the roots of affected trees, and proposed the name "phloem necrosis". In 1920 Stahel reported the disease to manifest itself in different forms, ranging from the acute wilting form ("rootrot") on one hand to the more gradual process of dieback of the twigs, yellowing of the leaves and ultimate death of the tree on the other hand. Between the acute and the chronic forms Stahel could find many intermediate stages. No direct relationship with soil- or climatic conditions could be established, while attempts to find aberrations similar to those occurring in the phloem necrosis of potatoes (Quanjer, 1913) were unsuccessful (Stahel, 1920).
Some years afterwards the relationship was investigated between the incidence of certain scale insects on the roots of coffee trees and the phloem necrosis disease (Reyne, 1924; Reyne and van Dijk, 1925). It was concluded that the disease was most probably transmitted by scale insects, a conclusion later opposed by Stahel (1934), but supported by Bünzli (1935). Fernandes (1928) suggested that poor soil conditions would induce the incidence of the phloem necrosis disease, but Stahel and Bünzli (1930) found on the contrary that the disease occurred mainly on well-drained, lighter soil.
In 1931 Stahel reported that in the phloem vessels of trees showing the phloem necrosis symptoms a characteristic multiple division of the sieve tubes occurred. In these abnormally divided cells organisms of a protozoan nature were observed. Stahel named the flagellate *Phytomonas leptovasorum* since it occurred only in sieve tubes. In 1932 and 1933 Stahel produced evidence that by root grafts the flagellates could be transmitted, and also the typical multiple division of the sieve tubes was induced in previously healthy trees. Here he could follow the migration of the flagellates to the main

branches. According to Stahel (1932) the symptoms of the disease could also be demonstrated in material from wilting Liberia-coffee trees in British-Guiana, while *C. arabica* material from Brazil revealed the presence of *P. leptovasorum* in that country. Work done on the Hemipteran insect-vectors of flagellates of laticiferous plant families (Holmes, 1925) induced Stahel (1934) to continue research along these particular lines, but the 2nd World War brought his work to an end. During this war the coffee fields were badly neglected and became overgrown by forest. When these fields were cleaned and brought back to production, after the war, a large scale wilting of coffee trees occurred. Van Suchtelen (1953) and Geijskes and van Suchtelen (1954) showed that the fungus *O. coffeae* (= *C. fimbriata*) was responsible for the epidemic. Since these authors assumed that the disease concerned was identical with the disorder investigated by Stahel, they stated that *C. fimbriata* was the true cause of phloem necrosis in *C. liberica.* No mention was made of any signs of multiple division of the phloem vessels in the trees investigated, a criterion explicitly stipulated by Stahel (1931) for the diagnosis of the phloem necrosis disease. Stahel (1954) reviewing his work, pointed out this obvious difference between the fungus disease, which was by then called "coffee canker", and the phloem necrosis disease. This led Van Emden to examine bark samples of coffee trees, showing the multiple division symptoms of the sieve tubes and in 1959 van Emden and van Suchtelen confirmed, that wilting of coffee trees could be caused by two completely different diseases, one being Stahel's phloem necrosis disease, the other being the woodrot caused by the fungus *C. fimbriata.*

Materials and methods

Environment

Most of the observations and investigations were made in two coffee fields, which were part of the "Peperpot" and "Waterland" estates, both near the Surinam river on well-drained soil. On both the plantations shade was provided by a canopy of *Erythrina glauca* (Willd.). Occasional observations in the coastal plain on the occurrence of phloem necrosis were carried out on eight other estates.

The climate in the coastal region of Surinam is tropical, with temperatures between 27° and 30 °C during daytime and between 23° and 27 °C during the night. Relative humidity varies between 70 % and 90 %. A long and a short dry season usually occur, but the climate is rather erratic. In the dry season a rather strong N.W.-trade wind prevails.

Microscopic examination of suspected trees

Strips of bark were cut from the roots, the main stem and the branches, and these strips were either examined in saline solutions or this material was fixed in Duboscq-Brasil's mixture in the field. Cross sections were examined for the presence of multiple division in the phloem. If this symptom was found, the outer bark of these samples was trimmed off and only soft tissue of the phloem near the cambium was embedded in paraffin wax for sectioning. Longitudinal sections at 8–12 μ were made; these were mordanted for 2 h and stained for the same time in Heidenhain's haematoxylin solution. After differentiation the sections were mounted in Canada balsam and examined at a × 1250 magnification.

Transmission experiments
In the graft transmission the following types of grafting were applied: leaf grafting, branch grafting, bark grafting and root grafting. For the latter method pieces of roots (20–25 cm length) showing the distinct multiple division of the phloem, were intergrafted in roots of healthy trees. All grafts were sealed with a graft wax. Where a hot water treatment preceded root grafting, this treatment was carried out for 25 min in a temperature controlled water bath.

Search for the vector
Hemipteran insects collected from the roots of coffee trees were fixed in Duboscq-Brasil's mixture to which chloroform had been added to facilitate penetration of the fixative. The salivary glands of these bugs were afterwards taken out and prepared for paraffin sectioning. Microtome sections of 6 μ were stained in Heidenhain's haematoxylin solution and mounted in Canada balsam.

Attempts to grow P. leptovasorum in pure culture
Attempts to grow *P. leptovasorum* in pure culture were made by using sterilized pecies of bark from roots or stems showing the multiple division of the phloem. These pieces were placed on a series of media: NNN, Casamino acids + glucose, yeast extract, various sugar solutions, coffee bark extract, coconut milk, Ringers-, Tyrode's- and Locke's solutions. Some other media consisted of latex aseptically collected from various lactiferous plants (*Euphorbia hirta* L., *Allamanda cathartica* L., *Poinsettia* (= *Euphorbia*) *pulcherrima* Grah., *Ficus elastica* Roxb., *Jatropha podagrica* Hook. and *Codiaeum variegatum* Blume), amended with benzylpenicilline B.P. to suppress bacterial contamination. These experiments were carried out at temperatures between 27° and 30 °C. No other temperature ranges could be tested because of lack of facilities. The pH of all solid and semi-solid media varied between 6 – 7.

Investigations of the possible role of nematodes
Goodey and Triffitt (1927) reported that nematodes could contain flagellates. Therefore it was deemed necessary to investigate the role of nematodes. Soil samples taken in coffee fields with a high, a low or no incidence of phloem necrosis were first investigated in Surinam, but later also sent to The Netherlands for more detailed examination at the Nematology section of the Laboratory for Phytopathology, Agricultural University, Wageningen, The Netherlands.

Soil analysis
Soil samples were also taken to search for a possible correlation between differences in any soil characteristic and the incidence of the phloem necrosis disease. Analyses of the samples were carried out by the Soil Chemistry section of the Agricultural Research Station, Paramaribo, Surinam.

Weeds
The weed flora of the two sites under investigation was examined by collecting plants throughout the year.

Results

Description of the flagellates
The occurrence of a flagellate of the trypanosomatid type was confirmed in a great number of longitudinal sections of root- and stem material of *C. liberica*, with the typical multiple division of the phloem vessels. The observed organism showed all the microscopical characteristics of the flagellates described by Stahel (1931).

External symptoms
It was observed that the first external symptoms of the phloem necrosis disease were bright, yellow leaves amidst normal green foliage. Under the tree the fallen yellow leaves were more numerous than under healthy trees. The roots of diseased trees were affected and showed die-back symptoms; gradually the symptoms became more severe and the tree died.
Stahel's (1920) observations, that different disease forms could occur, were confirmed. On one side of the range of forms is the chronic type of the disease in which three stages may be distinguished: the sparse yellowing and dropping of the leaves (stage I), increasing gradually, while the roots die back (stage II). 6–12 months after the onset only the young top leaves remain on the otherwise bare branches and all the roots are dead (stage III). The chronic type may be found all the year round. On the extreme side of the range the acute disease process can be found, in which the affected tree wilts and dies within 3 to 6 weeks. A few black leaves remain hanging from the bare branches. This type can mostly be found at the beginning of the dry season. In general the disease pattern is intermediate between these two extreme forms.
The first external symptoms are often similar to the symptoms of the *Ophiostoma* disease or "coffee canker", but soon the differences become apparent. In some cases it was observed that the two diseases occurred simultaneously in one tree. Obviously a tree which has already been weakened by phloem necrosis, is more easily invaded by the *Ophiostoma* fungus.

Anatomical evidence
Following the chronic disease pattern the first anatomical symptoms are those of stage I, when a multiple division of the phloem vessels occurs. The aberrant phloem is at this stage confined to a few cells near the cambium and is therefore not easily recognized (Fig. 1 and 2). In the longitudinal sections the strands of multiple-divided phloem cells are shorter and narrower than the normal phloem vessels and only a small number of big flagellates (spindle shaped, 14.0–18.0 μ × 1.0–1.2 μ, flagellum 6.0–8.0 μ) occur in the affected tissue (Fig. 3). The increased division of the phloem vessels leads gradually to an increasingly wider zone of smaller and shorter (1/4 × normal) cells, which in its abnormal structure contrasts sharply with the older tissue (Fig. 4, stage II–III). Meanwhile the flagellates wander out of the originally colonized zone, crowding the newly formed, multiple-divided sieve tubes. They become more numerous and smaller (spindle shaped, 4.0–14.0 μ × 0.3–1.0 μ, flagellum 1.0–5.0 μ; stage II). In the wake of this migration the leishmania-stage (2.0–3.0 μ) of the flagellates appear shortly afterwards in the areas first invaded (Fig. 5). The tissue which had shown the first symptoms of multiple division becomes necrotic and is now clearly distinguishable against the older, normal tissue. The adherence of the affected phloem tissue to the wood cy-

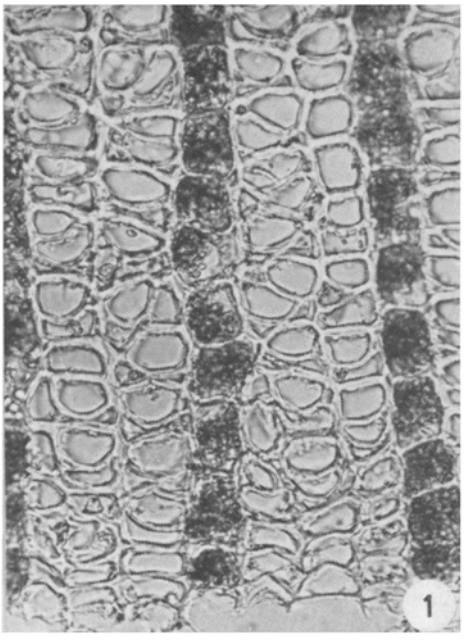

Fig. 1. *Coffea liberica.* Phloem of a healthy tree. Cambium cut away in cross section. (Photograph J. H. van Emden)

Fig. 1. Coffea liberica. Het floëem van een gezonde boom. Het cambium is weggesneden in de dwarse coupe. (Foto J. H. van Emden)

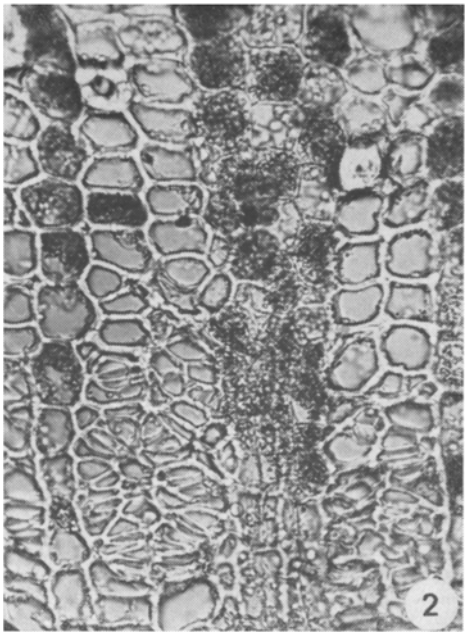

Fig. 2. *C. liberica.* Phloem of a diseased tree. Cross section of stage I in a slowly proceeding case. Multiple division still limited to a few cambial cells. (Photograph J. H. van Emden)

Fig. 2. C. liberica. Het floëem van een zieke boom. De dwarse coupe toont stadium I van een chronisch zieke boom. De multipele deling is nog beperkt tot enige cambiale cellen. (Foto J. H. van Emden)

linder becomes apparent, as the cambial activity stops. In the final stage of the disease (stage III) the sieve tubes are completely filled with the flagellates in a spaghetti-like packing, the individual, small and slender organisms (3.0–4.0 $\mu \times$ 0.1–0.2 μ) being hardly distinguishable in the stained longitudinal sections (Fig. 6).

The first appearance of flagellates in the sieve tubes is followed shortly afterwards by the first anatomical aberrations in the phloem and the external symptoms in the case of the slowly proceeding chronic disease pattern. The flagellates could in those cases be traced from the roots (stage I–II) upwards in the main stem (stage III), in which the multiple-divided zone of the phloem spread fan-like around the wood cylinder, reaching a height of about 2 m in the tree.

Unlike the aforementioned description of the chronic disease pattern, in acute cases the sequence of symptoms is accelerated. Around the whole base of the stem a narrow zone of multiple division can be found. In this narrow belt no flagellates are present, although the multiple division is very distinct. An anatomical comparison of the two extreme forms of the disease and the intermediate cases is given in Table 1, based of observations made on artificially infected trees. In no case have flagellates been observed outside the phloem vessels or in phloem tissue without the multiple division in the

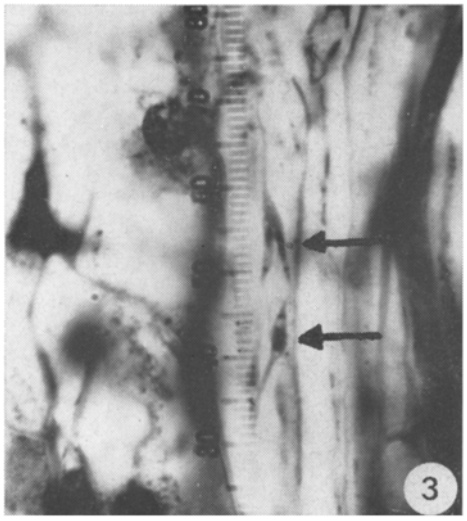

Fig. 3. *C. liberica.* Longitudinal microtome section (8μ) of the phloem of a diseased tree. Few, big flagellates (see arrows), one in a dividing stage (oil immersion × 950, 10 divisions = 8μ). (Photograph J. H. van Emden)

Fig. 3. C. liberica. Lengte-doorsnede van het floëem van een zieke boom (8μ). Slechts enige, grote flagellaten (zie pijltjes), waarvan één zich in een delingsstadium bevindt (olie immersie 950 ×, 10 schaalstreepjes = 8μ). (Foto J. H. van Emden)

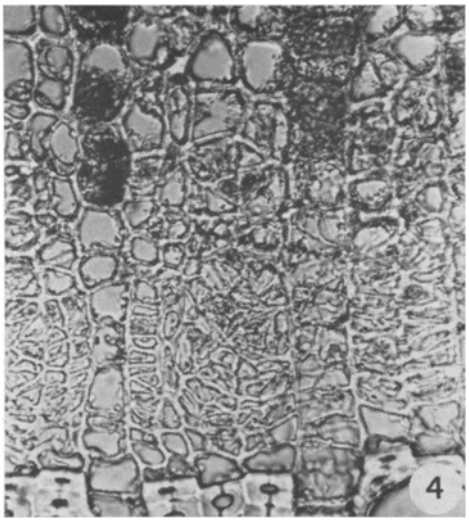

Fig. 4. *C. liberica.* Phloem diseased tree. Cross section of stage II–III. Multiple division sharply contrasting to the older normal tissue. Phloem firmly attached to the wood cylinder. (Photograph J. H. van Emden)

Fig. 4. C. liberica. Het floëem van een zieke boom, stadium II–III. De multipele zone onderscheidt zich duidelijk van het oudere weefsel. Floëem en xyleem blijven stevig aan elkaar vast zitten. (Foto J. H. van Emden)

Table 1. Disease patterns in artificially infected *C. liberica* trees in relation to the spread of flagellates and the anatomical aberrations of the phloem

Disease pattern	*First incidence of flagellates (weeks after infection)*	*Speed of the spread of the flagellates (in cm/week)*	*Number of rows of sieve tubes with MD**	*First external symptoms (months after infection)*	*Speed of decline of the tree (in months)*
Chronic	approx. 4–6	35– 40	2–8	4–6	approx. 8–15
Intermediate	4–6	60– 80	3–5	4–5	5– 8
Acute	4–6	100–120	1–3	3–4	½– 1½

* MD = multiple division of the phloem

Tabel 1. Het ziekteverloop in kunstmatig geïnfecteerde C. liberica bomen in verband met de verspreiding van de flagellaten en het optreden van anatomische afwijkingen van het floëem

adjacent cells. Ordinarily the flagellates migrate vertically upwards in the phloem and horizontally through the lateral sieve plates into healthy sieve tubes. A downward movement into unaffected roots has, however, been observed in the chronic disease cases.

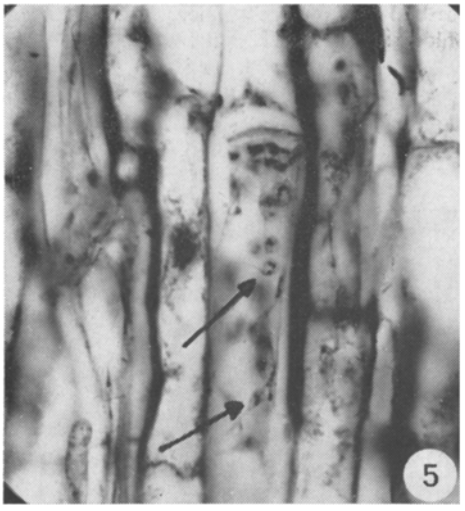

Fig. 5. *C. liberica.* Longitudinal microtome (8μ) of the phloem of a diseased tree. The first leishmania forms (see arrows) appear in stage II (oil immersion × 950). (Photograph J.H .van Emden)

Fig. 5. C. liberica. Lengte-doorsnede (8μ) van de zeefvaten van een zieke boom. De eerste leishmania vormen (zie pijltjes) verschijnen in stadium II (olie immersie 950 ×). (Foto J. H. van Emden)

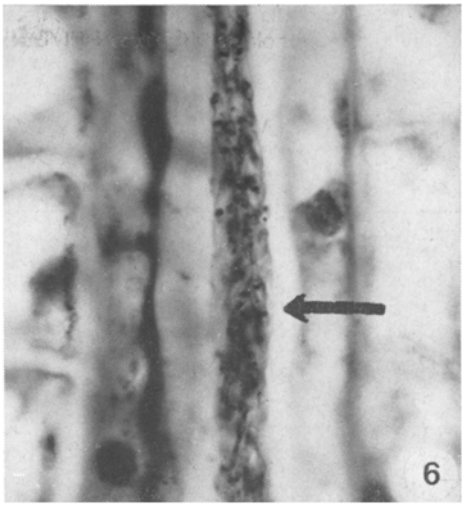

Fig. 6. *C. liberica.* Longitudinal microtome section (8μ) of the phloem of a diseased tree. Numerous flagellates (see arrow) in the final stage of the disease ("spaghetti flagellates"); the individual, small and slender organisms are hardly distinguishable (oil immersion × 950). (Photograph J. H. van Emden)

Fig. 6. C. liberica. Lengte-doorsnede (8μ) van de zeefvaten van een zieke boom. Talrijke flagellaten (zie pijltjes) zijn waarneembaar in het laatste stadium van de ziekte ("spaghetti flagellaten"); de organismen zijn nauwelijks individueel waarneembaar (olie immersie 950 ×). (Foto J. H. van Emden)

Epidemiology

By careful observations of plots in which all trees were numbered, evidence could be obtained that the disease spread from one tree to another, in this way gradually infecting large areas of the fields. In most cases it could be ascertained that a diseased tree had been planted on or near a place where previously a case of phloem necrosis had occurred (Fig. 7).

The disease symptoms are not manifest on young trees in the field, until the trees have carried a crop for 2 years. Only healthy, well developed trees are apparently prone to the phloem necrosis disease, while in weak and slowly growing trees the disease has never been found.

The spread of the disease in the field is not correlated with the prevailing wind.

Transmission experiments

Green branches, leaves, pieces of bark from the stem and roots of diseased trees were grafted in various series of experiments onto healthy trees. Although branch and leaf grafts succeeded readily, no transmission of the disease was ever observed, not even

Fig. 7. Spread of the disease in three years time in a section of "Peperpot" estate. Affected trees can be found on or near places where previously cases of phloem necrosis had been uprooted.

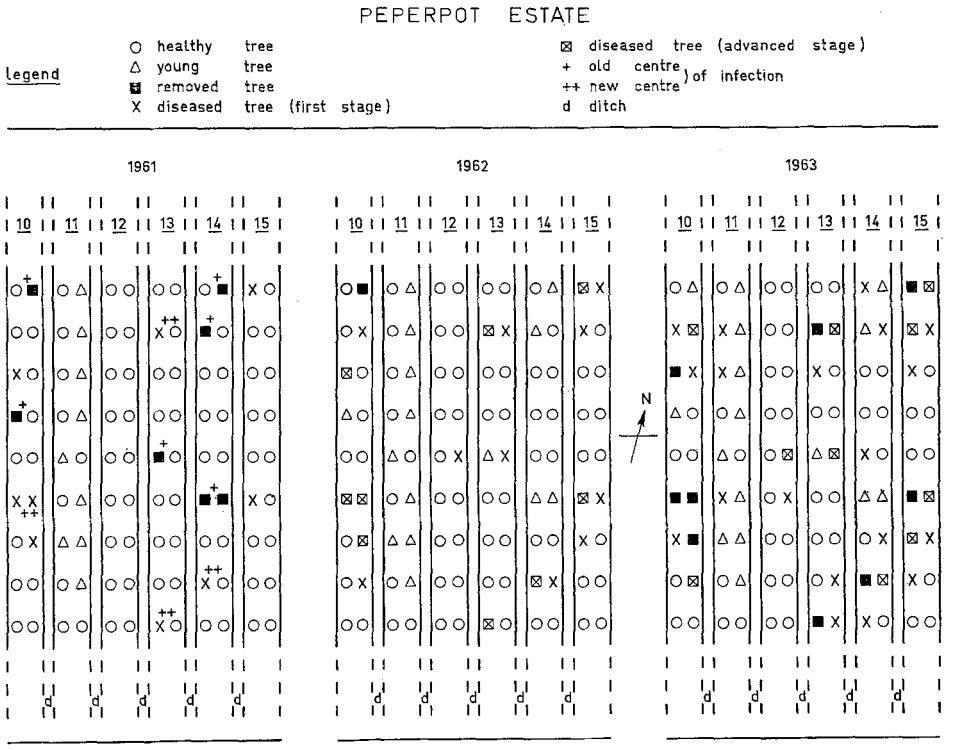

Fig. 7. Verspreiding van de ziekte in drie jaren in een gedeelte van "Peperpot" plantage. Aangetaste bomen kunnen worden gevonden op of bij plaatsen waar eerder gevallen van de zeefvatenziekte waren opgerooid.

after $1\frac{1}{2}$–2 years. As in green branches and in leaves no multiple division of the phloem is to be found, this result suggests that the pathogen does not occur in the tree outside the areas with multiple division.

The grafts of pieces of bark only succeeded when multiple division did not yet occur, because when multiple division had commenced, the bark could not be easily separated from the wood cylinder and then the cambium was so much damaged that a bark graft did not succeed. By bark grafting no transmission of the disease was obtained.

In "Peperpot" estate eight healthy, fruit bearing trees were grafted, six trees with root material from diseased trees, two with material from healthy trees. After 4 to 6 months five trees of the former group were showing the first external and internal symptoms of the disease. Of these five trees one followed the acute disease pattern, three trees succumbed to the chronic type, while the last tree of this group recovered after the grafted roots were cut off near the stem base of the tree immediately after the first external symptoms. The trees grafted with healthy root material did not show any symptoms.

Attempts were also made to infect young coffee trees by using roots from affected trees. First six young trees planted in drums were grafted; four with diseased root material,

one with healthy material and one was left untreated. Of the four inoculated trees only one showed the external and internal symptoms after 8 months. After 10 months another young tree showed the same symptoms, but in both cases the disease process did not seem to intensify even after two years. The other trees did not show any symptoms after 2 years.

In a second attempt eleven young trees, planted in a plot at the Agricultural Research Station, Paramaribo, were grafted with diseased root material and four other trees with healthy root material. Of the eleven trees three showed the external and internal symptoms of the phloem necrosis disease after 7 months, while three others gave symptoms after 10 months. Five trees of this group and the four control trees remained healthy. Although flagellates were observed in the trees with the disease symptoms, these trees were still alive after 2 years. An identical series of grafts was carried out on six young trees at "Peperpot" estate. Four were grafted with diseased root material, two with healthy material. Of the former group three showed the first symptoms after about 6 months. The control trees remained healthy. Again the disease seemed to be

Table 2. Transmission of the phloem necrosis disease from *C. liberica* to other *Coffea* species by root grafting

Coffea species grafted october 1961	*December 1961*	*April 1962*	*July 1962*	*General remarks*
C. abeocuta	—	—	—	tree healthy
C. arabica	±	+	+	yellow leaves, wood attached to phloem; disease process slow and fatal
C. excelsa	—	+	+	yellow leaves, disease process not distinct
C. stenophylla	+	+	+	many yellow leaves, wood attached to phloem
C. canephora	±	±	±	no external symptoms
C. robusta	±	±	±	no external symptoms
C. spec. (?)	—	—	—	no symptoms
C. canephora (P.B. 42)	±	±	±	wood attached to phloem, no external symptoms
C. canephora (P.B. 39)	—	—	—	tree healthy
C. canephora (P.B. 39)	—	—	—	tree healthy
Clone 203 (+)	—	—	—	tree healthy
Clone 218 (++)	—	—	—	tree healthy

— = no multiple division of the phloem
\+ = a distinct multiple division of the phloem
± = multiple division of the phloem less marked

(+) = *C. congensis* × *C. canephora*
(++) = *C. canephora* × *C. congensis*

Tabel 2. De overdracht van de zeefvatenziekte van C. liberica op andere Coffea soorten door middel van wortelenten

non-fatal on artificially infected, young trees even after 2 years, while on the whole the symptoms tended to diminish in this period.
In the museum plot of the Agricultural Research Station ("La Poule" estate) one tree of each available *Coffea*-species was grafted with root material of *C. liberica* trees, showing the typical symptoms of the phloem necrosis disease. The results of this experiment are shown in Table 2. It can be seen, that especially *C. excelsa*, *C. arabica* and *C. stenophylla* showed the unmistakable multiple division of the phloem after 8 to 12 months, while *C. canephora* and *C. robusta* displayed less marked aberrations of the phloem. Flagellates could be found in the first group of *Coffea*-species with a clear multiple division. The external symptoms, however, were less noticeable than in *C. liberica* and apparently the disease was not or less fatal to these three species, as all trees were still alive after one year.
The root grafting experiments were further extended by applying a hot water treatment to affected root material before grafting this material onto healthy trees. In this way information has been obtained on the possibility of transmitting the sidease after root material had been exposed to temperatures of 40°, 45° and 50°C for 25 min. The results of this experiment are presented in Table 3. No transmission occurs after the 50°C-treatment, as all the root grafts died even of the control. Two out of ten trees showed symptoms after the 45°C-treatment; siks out of eleven trees after the 40°C-treatment. The root material not subjected to a treatment, induced disease symptoms in eight of the ten trees of this group.

Table 3. Transmission of the phloem necrosis disease to healthy *C. liberica* trees by rootgrafting after a heat treatment

Number of treated trees	*October 1961*		*Multiple division of the phloem*									*Remarks*
	rootgraft	*heat treatment (°C)*	*Jan. 1962*			*April 1962*			*July 1962*			
			—	±	+	—	±	+	—	±	+	
10	diseased	—	8	2		3	5	2	2	2	6	+ trees showing typical symptoms
11	diseased	40	7	4		7	3	1	5	2	4	most grafts in trees dried up
10	diseased	45	10			8	2		8	2		ditto
6	diseased	50	6			6			6			all grafts dried up
8	healthy	—	8			8			8			all grafts took, trees healthy
4	healthy	40	4			4			4			all trees healthy, some grafts dried up
4	healthy	50	4			4			4			all grafts dried up

— = no multiple division of the phloem ± = multiple division of the phloem less marked
\+ = a distinct multiple division of the phloem

Tabel 3. Overdracht van de zeefvatenziekte op gezonde C. liberica bomen door middel van wortelenten na een warmtebehandeling

Possible transmission by insects

Insects were collected from the roots of diseased and healthy coffee trees. These insects proved to belong mostly to the *Pentatomidae*, genus *Ochlerus.* Microtome sections of the salivary glands did not show any stages of the life cycle of *P. leptovasorum.* In some cases it could be established, that certain flagellates occurred in the midgut of these insects, but these organisms might have been normal components of the intestinal fauna.

Scale insects could be found on the roots of a *Caladium*-species, a common weed in the coffee plantations. Only rarely these insects were found on the roots of coffee trees. A great number of scale insects was squashed in physiological salt solution, but flagellates were never observed.

No other insects could be found in significant numbers on or near coffee trees to indicate a relationship with the phloem necrosis disease.

Possible transmission by nematodes

The nematodes population survey did not reveal any differences in the population of nematodes around diseased and healthy trees. The number of parasitic nematodes was on the whole low. In squashed preparations of these parasitic nematodes (fixed material) no flagellates could be found.

Soil conditions

As a consequence of Kuyper's (1913) and Fernandes' (1928) suggestions, that certain soil conditions would induce the phloem necrosis disease, soil samples were taken at different times of the year from various plots at "Peperpot", "Waterland", "Ma Retraite" and "La Poule" estates. The samples were taken from plots with high, a low or no incidence of the phloem necrosis disease.

It was found, that the disease only occurred on plots some distance away from the river, where the soil had a lighter structure and was well drained. Near the river, where periodically flooding occurs, no phloem necrosis could be found. These observations agree with those of Stahel and Bünzli (1930).

Climate and environment

The climate in the coastal region of Surinam is uniform and no major climatic differences could be found between coffee estates with or without the phloem necrosis disease. The symptoms of the disease become apparent during the dry season, since in the rainy season the roots of affected trees can still take up enough water to maintain a reasonably healthy appearance. Therefore no direct role of the macro-climate on the incidence of the disease can be suggested. However, the micro-climate may have an indirect effect on the disease vector.

Most coffee trees in Surinam are grown under shade trees for which purpose mostly *Erythrina glauca* (Willd.) trees are used. Where shade trees are absent the coffee looks yellowish and in poor condition, but no phloem necrosis has been found in such fields. In all fields with a high incidence of the disease the shade tree canopy was invariably rather dense. It appears therefore, that shade trees have a direct effect on the general condition of the coffee trees and an indirect effect on the disease incidence, by inducing a more favourable micro-climate for the possible disease transmittor.

Weeds

A survey of the weed flora in the two experimental sites showed that in "Peperpot" estate *Wedelia trilobata* (L.) Hitsch., *Commelina nudiflora* L. and *Pothomorpha peltata* Miq. were dominant weeds, while in "Waterland" estate *W. trilobata* and *Brachiaria purpurescens* Henr. were dominant. In none of the weeds mentioned could the presence of a flagellate be demonstrated. The only species in which flagellates could be found was *Euphorbia hirta* L. These organisms were identical with *Phytomonas* (= *Leptomonas*) *davidi*, described by Lafont (1909) in *E. pilulifera* L. (= *hirta*) and mentioned by Stahel (1931). Although *E. hirta* is a very common weed in the coastal region, no further relationship between the incidence of flagellates in this weed and the phloem necrosis disease could be found.

Search for other pathogens

The relationship between the phloem necrosis disease and the incidence of certain bacteria has been investigated. In coffee trees in Surinam one bacterial disease (van Hoof, 1962) has been recorded, but the pathogen, *Erwinia coffeae* van Hoof, only affects the wood cylinder of the coffee tree and the symptoms are quite different from those caused by the phloem necrosis disease. Both diseases can occur, though only very rarely, on the same three. Surface sterilized pieces of coffee bark, containing flagellates, do not give a bacterial growth on sterilized media. It seems unlikely that bacteria are involved in the phloem necrosis disease.

Experiments on hot water treatment before grafting root material of diseased trees onto healthy trees have been set up also with the idea of evaluating the role of viruses in the disease complex (van Hoof, personal communication, 1961). The results (Table 3) only indicate, that root material apparently cannot stand temperatures of 50°C for 25 min, as both the grafts of the diseased and healthy material desiccated in all cases. Although the disease can be transmitted after treatments in the range of 40°–45°C, the total failure of all treatments at 50°C does not allow any suggestions with regard to the role of viruses. Further investigations showed that no sap transmission could be induced, using sap from roots, bark and leaves inoculated onto cucumber, tobacco and bean plants. Electron-micrographs of sap of roots, bark and leaves of diseased trees did not reveal any virus-like particles, as compared to healthy material. The role of viruses is not yet investigated exhaustively, but there are no indications that they are involved in the phloem necrosis disease.

With regard to the fungi the role of *C. fimbriata* in coffee canker (van Suchtelen, 1953; Geijskes and van Suchtelen, 1954; van Emden, 1962) has already been mentioned. This disease occurs in the xylem of the coffee tree: anatomical aberrations of the phloem have never been observed. Here the disease develops much slower than with phloem necrosis, because the fungus lives in the xylem, whereby the flow of assimilates inside the phloem remains unaffected. Phloem necrosis and coffee canker can occur together on the same tree, but mostly they are found on separate trees. Coffee canker is to be found on plots where growing conditions are unfavourable; phloem necrosis on well-kept and well-drained soils, where trees are vigorous.

Neither direct nematode damage in roots of diseased trees could be observed, nor were nematodes ever found in root tissue of diseased trees.

Attempts to grow P. leptovasorum in pure culture

Initial attempts were made with *P. davidi*, as this organism was readily available. Droplets of latex were kept under observation for some time and it was found that the flagellates became distorted after 3–4 h, while the number of bacteria increased greatly. The flagellates were completely dead after 5–6 h. The same was observed when *P. davidi* was maintained in physiological salt solutions. In another experiment *E. hirta* plants were surface sterilized; droplets of the latex containing flagellates were then transferred to sterile culture media (malt agar, potato dextrose agar, NNN-medium and yeast extract). None of the flagellates showed any signs of life after 1–3 h.

With *P. leptovasorum* it was more difficult to get the organisms out of the phloem vessels. In physiological salt solutions the flagellates could be found in the solutions after keeping pieces of coffee bark in it for some time. The flagellates lost their mobility after a period of $1\frac{1}{2}$–2h. In all other media used the flagellates lost their mobility after the same period.

Present records of phloem necrosis in other countries

C. liberica material received from British-Guiana showed the marked multiple division of the phloem vessels typical for the disease. As the material had been fixed in alcohol (Stahel, 1931), there were no flagellates to be seen. Considering the consequent relationship between multiple division and the incidence of *P. leptovasorum*, it may be concluded that the phloem necrosis disease must still be present in Guiana, taking into account the external symptoms described already by Stockdale (1909). More recent information on the presence of the disease in Brazil on *C. arabica* (Stahel, 1932) could not be obtained. Perhaps are the losses due to the disease so negligible that they attract no attention in that country, which agrees with the less marked disease symptoms obtained on root grafted *C. arabica* trees in Surinam. No recent information was received from Colombia and San Salvador (Stahel, 1954) or Liberia, the home country of *C. liberica.*

Discussion

Research work from 1960 till 1963 has shown that the observations made by Stahel were correct. Flagellates similar to those described by Stahel could be found, always in connection with a multiple division of the phloem vessels. The various transmission experiments have shown that the disease cannot be graft-transmitted by parts of the diseased plants not showing the multiple division of the phloem. In the case of bark grafts the transmission failed because the cambium was damaged while removing the material from the donor tree. Only root grafts gave successful transmission under various conditions.

Young coffee trees could be infected artificially by root grafting, but the symptoms induced were less conspicuous and the disease apparently not fatal to the tree. Three other *Coffea* species could also be infected artificially by root grafting; the symptoms were again less marked and the disease process less often fatal then on *C. liberica.* In all these cases of a successful transmission by root grafting it was established that the diseased root material showed multiple division of the phloem and still had an active cambium. It seems therefore likely that the flagellates associated with the anatomical aberration of the phloem are the incitement of the disease. This is supported to some

extent by the hot water treatment preceding grafting. Harvey and Lee (1943) reported the sensitivity of latex flagellates to temperatures higher than 40 °C. Whether this applies to the coffee flagellates has not been established satisfactorily, considering the negative results of attempts to grow the flagellates in pure culture. In the range of temperatures applied before grafting, however, a good transmission rate had been achieved after 40 °C (approx. 45 %), a lower transmission rate after 45 °C (approx. 18 %) and no transmission after 50 °C. This would suggest an effect on the flagellates in the temperature range 40 °–45 °C, although it is possible that the cambial tissue has also been damaged, which might explain the failure of all grafts at 50 °C.
No indication was found that other pathogens may play a role in the phloem necrosis disease. Although the work done on viruses – with special reference to the transmission experiments after hot water treatment – and nematodes cannot be considered exhaustive, such pathogens nor fungi and bacteria are likely to be involved. As long as coffee flagellates cannot be grown in pure culture, the ultimate proof of the pathogenicity of *P. leptovasorum* will remain unobtainable.
The disease seems to occur only on the light, well-drained and well-kept fields. The vector may be an insect, which prefers these soils and micro-climatic conditions to the heavy, wet and poorer soil conditions of the coffee fields, where only the coffee canker occurs. Further work on the salivary glands and the mid-gut of the Hemipteran insects found on the coffee roots, should be done, if possible on live material (Vickerman, 1962). Considering the role of *C. liberica* on the world market and the relatively low losses due to phloem necrosis, it seems doubtful that research on this disease will be further pursued, but the ultimate proof of the relationship between *P. leptovasorum* and the phloem necrosis disease is still of scientific interest.

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Samenvatting

Onderzoekingen naar de oorzaak van de zeefvatenziekte van Coffea liberica in Suriname, Zuid-Amerika

Het voorkomen van de flagellaat *Phytomonas leptovasorum* Stahel in het floëem van

bomen van *Coffea liberica*, die de typische verschijnselen van de zeefvatenziekte vertonen, is opnieuw aangetoond (Tabel 1). De flagellaat komt alleen voor in bomen met een abnormale deling (= multipele deling) van de zeefvaten (Fig. 1–6). Deze anatomische afwijking is kenmerkend voor de floëemnecrose en treedt niet op bij de verwelkingsziekte, veroorzaakt door de schimmel *Ceratocystis fimbriata* (= *Ophiostoma coffeae*). Het onderzoek naar een mogelijke rol van virussen, nematoden en bacteriën heeft geen positieve resultaten opgeleverd.
De ziekte komt in de natuur alleen voor in volwassen bomen (Fig. 7), maar het is opnieuw mogelijk gebleken om door middel van wortelenten de ziekte op jonge bomen over te brengen (Tabel 2 and 3). De ziekteverschijnselen zijn dan echter minder extreem en het ziekteverloop niet fataal. Ook andere *Coffea*-soorten kunnen zowel natuurlijk als kunstmatig door de floëemnecrose worden aangetast. De verschijnselen zijn minder opvallend en de aangetaste bomen schijnen zich na verloop van tijd te herstellen.
Het is nog onbekend op welke wijze de ziekte in de natuur wordt overgebracht. Het vermoeden bestaat dat een insekt van de Hemiptera-groep de overbrenger is, aangezien deze insekten *Phytomonas* (= *Leptomonas*) *davidi* in *Euphorbia* spp. kunnen overbrengen. Pogingen om de flagellaten in reincultuur te kweken zijn mislukt. Het uiteindelijke bewijs dat *P. leptovasorum* het pathogeen is, kan daarom nog niet worden geleverd.

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