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A CONTRIBUTION TO THE KNOWLEDGE
OF *SPONGOSPORA SUBTERRANEA* (WALLR.)
LAGERH., THE CAUSE OF POWDERY SCAB
OF POTATOES

BIBLIOTHEEK
DER
LANDBOUWHOOGESCHOOL
WAGENINGEN.

A. P. KOLE

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Dit proefschrift met stellingen van

ARIE PIETER KOLE

landbouwkundig ingenieur, geboren te Wageningen, 15 October 1920, is goedgekeurd door de promotor Dr A. J. P. OORT, hoogleraar in de plantenziektenkunde.

De Rector Magnificus der Landbouwhogeschool,
W. J. DEWEZ

Wageningen, 12 December 1953

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MET EEN SAMENVATTING:

BIJDRAGE TOT DE KENNIS VAN *SPONGOSPORA SUBTERRANEA*
(WALLR.) LAGERH., DE VERWEKKER VAN POEDERSCHURFT BIJ
AARDAPPELEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR W. J. DEWEZ,
HOGLERAAR IN DE LANDBOUWPLANTENTEELT,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
DER LANDBOUWHOGESCHOOL TE WAGENINGEN
OP WOENSDAG 27 JANUARI 1954 TE 16 UUR

DOOR

ARIE PIETER KOLE



H. VEENMAN & ZONEN - WAGENINGEN - 1954

De inhoud van dit proefschrift verschijnt tevens in het Tijdschrift
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STELLINGEN

I

Het is wenselijk aan de eis van de Nederlandse Algemene Keuringsdienst, krachtens welke in het af te leveren stamselectiemateriaal geen poederschurft mag worden aangetroffen, de bepaling toe te voegen, dat bij niet meer dan zeer sporadisch voorkomen van de aantasting, de partij op verklaring mag worden afgeleverd in het betrokken keuringsgebied.

II

Door de waarnemingen van KUSANO is niet bewezen, dat de aanwezigheid van zoösporen met meer dan twee flagellen bij *Olpidium viciae* KUS. het gevolg is van het achterwege blijven van de laatste deling bij hun ontstaan.

KUSANO, S. – 1912, J. Coll. Agr. Imp. Univ. Tokyo 4:141–199.

III

De huidige kennis omtrent de Plasmodiophorales impliceert, dat alle beschouwingen betreffende geslachtelijke differentiatie, homo- en heterothallie, opeenvolging van haploïde en diploïde generatie etc., bij deze orde een sterk speculatief karakter dragen.

KARLING, J. S. – 1942. Plasmodiophorales.

IV

Het handhaven van de naam *Polyspora lini* LAFF. is te verkiezen boven de wijziging in *Pullularia pullulans* var. *lini*, zoals door WHITE wordt voorgesteld.

WHITE, N. A. – 1945, J. Council Sc. Ind. Res. 18:141–149.

V

Inhibitoren van plantenviren werken niet rechtstreeks op het virus, maar via de plant.

BAWDEN, F. C. and FREEMAN, G. G. – 1952, J. gen. Microbiol. 7: 154–168.

VI

Onderzoek naar eventueel giftige werking van bestrijdingsmiddelen op bijen behoort niet in vitro te geschieden maar door gebruikmaking van een normaal bijenvolk.

VII

De verklaring, die MORDVILKO geeft voor het voorkomen van Fordinen in gebieden waar hun primaire waardplant ontbreekt, is zeer aanvechtbaar.

MORDVILKO, A. – 1935, Ergebnisse und Fortschritte der Zoologie 8: 36–328.

VIII

Het is wenselijk het woord „promitose” te handhaven voor de somatische kerndeling bij de Plasmodiophorales.

IX

Hetgeen bij de Plasmodiophorales o.a. dikwijls als „akaryotisch stadium” is beschreven, maakt waarschijnlijk deel uit van de prophase van de op dat stadium volgende kerndeling.

X

Het gebruik van vastvleugelige vliegtuigen ter bestrijding van ziekten en plagen van Landbouwgewassen is niet verantwoord.

XI

Alhoewel bij het veredelen van landbouwgewassen in Nederland het kweken op resistentie tegen parasieten reeds een belangrijke plaats inneemt, dient meer aandacht te worden besteed aan het kweken op resistentie tegen die parasieten, waarvan de aanwezigheid in ons land nog niet is geconstateerd, maar die toch een bedreiging voor de teelt en de export kunnen vormen.

XII

Voor het scheppen van één Benelux-markt voor zuivelproducten is het een eerste voorwaarde, dat de eisen ten aanzien van de kwaliteit van de melk en de daaruit bereide producten in de betrokken landen worden geuniformeerd.

XIII

Voor zover de werkzaamheden het toelaten en de arbeidsproductiviteit er niet onder hoeft te lijden, verdient, met behoud van het aantal weekuren, de werkweek van 5 dagen ook in ons land de voorkeur boven die van 5½ dag.

Aan de nagedachtenis van mijn Ouders

Aan mijn Vrouw

Aan Ellen

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GENERAL INTRODUCTION

The occurrence of Powdery Scab has become important in the export of potato tubers from the Netherlands for, prior to their importation into other countries, they must generally satisfy the plant health requirements laid down by the importing country as a condition of their entry. These requirements induced the Phytopathological Service (Plantenziektenkundige Dienst) to approach the Board of Agriculture (Directie van de Landbouw) with a view to investigating the possibility of controlling Powdery Scab and the factors that influence the development of the infection during storage. Eventually the author was presented with this problem to study. From 1st February, 1949, to 1st January, 1950, facilities were kindly provided by the Laboratory of Mycology and Potato Research (now, Laboratory of Phytopathology) of the Agricultural University. From 1st January, 1950, the study was continued in the newly founded Institute for Phytopathological Research, and here they were continued by the writer during 1952.

The occurrence of Powdery Scab in the Netherlands is mainly limited to the sand and sandy-peat soils in the North-Eastern part of the country. The severity and extent of the disease vary yearly, for the incidence of the disease is influenced by weather conditions. In dry summers the disease is rare and in normal or very wet summers the disease may become serious. In other parts of the country the disease only occurs sporadically and is not severe.

Powdery Scab is important in the export of potato tubers but the problem is of minor importance in tubers destined for home and industrial consumption. However, tubers affected with Powdery Scab may decay from invasion by secondary organisms, when a heavy loss may occur depending upon the length of time of storage.

Seed potatoes and potatoes for consumption infected with Powdery Scab are mostly unsaleable at home and, as already mentioned, abroad where, either no tolerance or only a very slight tolerance to the degree of infection present, is allowed.

Attempts were first made to investigate the disease in the field, but on account of the large fluctuations in the occurrence of the disease found from year to year, the investigations were later transferred to pot experiments in glass-houses where control over the conditions for infection was possible.

Pot infection experiments based on the experience of previous workers were unsuccessful and a thorough investigation of the factors governing infection was commenced together with detailed studies of the fungus life history.

Part I describes the morphology, cytology and life history of the fungus, Part II describes the relationship of environmental factors to infection. The study of the factors that influence infection during storage is included in Part I. Methods of Powdery Scab control are still under investigation.

PART I

THE MORPHOLOGY, CYTOLOGY AND
LIFE HISTORY OF THE FUNGUS

CHAPTER 1

THE ZOOSPORANGIUM AND ZOOSPORES

A. Introduction

The life cycle of *Spongospora subterranea* (WALLR.) LAGERH. may be briefly summarized as follows:

The fungus hibernates as spore balls in powdery scab lesions occurring on potato tubers, and also as free spore balls in the soil. The spore ball consists of a spongy aggregate of resting spores, each of which produces a single zoospore at germination. The zoospores penetrate the epidermal cells and root hairs of the young host roots and eventually develop into small groups of zoosporangia. Germinating zoosporangia release zoospores which (perhaps after fusing in pairs) infect potato plants with the subsequent development of powdery scab lesions on the tubers and warts on the stolons and the roots. The resting spores develop and mature in the lesions and warts and are eventually liberated following the decay of the affected tissues.

The zoosporangial stage of the fungus was first discovered by LEDINGHAM (1935). He regarded the development of the zoosporangium as resulting from a process of budding, in contrast to zoosporangial development in related genera where previous workers have shown plasmodial segmentation to occur. LEDINGHAM also observed binucleate, quadriflagellate zoospores, though whether this condition indicated fusion of gametes or incomplete separation of zoospores within the zoosporangia remained undetermined.

In this chapter the zoosporangial stage in the life cycle of the fungus is re-examined, with particular reference to the two points investigated by LEDINGHAM.

B. Materials and methods

Infected roots from the soil were found to be unsuitable for microscopical studies. The straggling form of the roots made it difficult to get good microtome sections, while whole mounts could not be stained satisfactorily with haematoxylin and rendered the slides too thick for use with immersion objectives. These difficulties were overcome by sectioning the straight root-tips bearing root hairs obtained from potato and tomato plants grown in nutrient solutions inoculated with the fungus. The „eyes” of potato tubers were cut out together with about 1 cc. of the surrounding tissue and planted in boxes of steam-sterilized, garden soil. When the shoots were 3–4 cm. high, the young plants were removed from the soil, rinsed with tap water and transferred to nutrient solutions. The tomato seedlings were grown from seed, but were other-

wise treated identically. The plants were first grown in VON DER CRONE'S solution, as modified by MES (1930), (pH 6.0), but owing to the presence of insoluble salts VON KOPETZ & STEINECK'S (1949) nutrient solution (pH 6.2) was later preferred. The trace element compounds NiSO_4 , $\text{Co}(\text{NO}_3)_2$, TiO_2 , LiCl , SnCl_2 , and MoO_3 and also the sulphuric acid constituents of the latter solution were omitted.

The nutrient solutions were inoculated with spore balls in the following manner. Powdery scab lesions were scraped from diseased potato tubers and the scrapings dried and pulverised in a mortar. The powder obtained was passed through a $50\ \mu$ mesh sieve to eliminate coarse particles and a small quantity was then added to the nutrient solutions. When the root hairs were infected, root-tips with root hairs were cut off and fixed in FLEMMING'S solution (the weak solution according to RAWLINS (1931)). The material was dehydrated using tertiary butyl alcohol (JOHANSEN, 1940), imbedded in paraffin-wax and sectioned with a rotary microtome at thicknesses varying from 10–16 μ . The majority of the sections was stained with HEIDENHAIN'S haematoxylin and in order to study details of the nuclei, some sections were stained by NEWTON'S iodine gentian-violet method. Transverse and radial sections were used for the study of the zoosporangia in the root hairs and tangential and radial sections were used for the study of zoosporangia in the epidermal cells. Whenever observations were possible, the development of the fungus in roots grown naturally in infected garden soil was found to be identical with the development of the fungus in roots grown in nutrient solutions.

The examination of slides of prepared sections was made with a bright field microscope ¹⁾. Some observations on living roots in water mounts were made using a phase contrast microscope ²⁾.

Zoospore discharge from the zoosporangia was first studied using infected roots of small potato plants grown from „eyes” in the manner described, after growing the plants in soil artificially heavily infected with the fungus. Later experiments showed that tomato seedlings were more suitable for these observations and they were used in all subsequent work. Tomato seedlings bearing the first pair of true leaves were transferred to heavily infected soil, and zoospore discharge was studied when microscopical observations revealed the presence of mature zoosporangia in the root hairs. It was found that zoospore discharge could best be observed soon after mounting roots containing zoosporangia in water. The following technique usually resulted in a fairly good discharge (Plate II, fig. 22). After lifting from the soil, the roots were washed with a fine, strong jet of water to remove the majority of adhering soil particles. The roots were then transferred to a petri dish containing a shallow layer of water. Using a hand-lens, very thin rootlets bearing root hairs were cut off, about 1 cm. long, and mounted in water. The best results were obtained when the soil in the pots containing diseased plants was not watered during the preceding twenty-four hours. In this way the germination of mature zoosporangia was retarded long enough to permit good zoospore discharge immediately the rootlets were mounted in water. Occasionally zoospore discharge and zoospores obtained in

¹⁾ ZEISS-WINKEL Standard-Microscope, obj. $10 \times$ n.a. 0.25, $40 \times$ n.a. 0.65, H.I. $100 \times$ n.a. 1.30 and photo-oculars $12 \times$ and $18 \times$.

²⁾ BAUSCH & LOMB Phase Contrast Microscope, pos. contr., obj. $21 \times$ n.a. 0.50, $43 \times$ n.a. 0.65, H.I. $97 \times$ n.a. 1.25 and ocular $10 \times$.

water mounts were examined microscopically sometimes after lightly staining with a weak aqueous solution of neutral red. However, most zoospore observations were made with the phase contrast microscope on unstained zoospores. For cytological and morphological observations, suspensions of zoospores were prepared on glass slides, killed by exposure to the fumes of 1 % osmic acid and stained. For morphological details, the zoospores were stained with a modified LÖFFLER's stain (COUCH, 1941). After staining, the slides were allowed to dry in an inverted position in order to obtain an even distribution of zoospores (ELLISON, 1945). The dried slides were mounted in Canada balsam. For cytological purposes the zoospores were stained according to COTNER (1930) and were either examined at once in the diluted stain or mounted in Canada balsam after drying and differentiation in clove oil.

C. The zoosporangial stage

The youngest stages of infection

Plasmodia were seen to develop in the young root hairs and epidermal cells from uninucleate, spherical zoospores ¹⁾ (Plate I, figs. 3 and 4). In older cells the zoospores developed either poorly or not at all. The zoospores, after penetrating into the cells, flattened against the cell walls (Plate I, fig. 5) and developed into plasmodia which, lying close together, may sometimes have coalesced. The number of zoospores entering the root hairs and epidermal cells varied. As many as eight have been observed in one root hair and in some of the older epidermal cells still more have been seen. In these instances, there were indications, however, that zoospores may have entered freely through damaged root hairs. Zoospores that have penetrated into the host cells were spherical, vacuolated and contained several highly stainable granules and „wheel-type” nuclei. The granules gradually disappeared and the cytoplasm became finely reticulated. The zoospores, both before and after penetration, measured $4.77 \pm 0.15 \mu$ and $4.54 \pm 0.15 \mu$ in diameter respectively. Plate I, fig. 1, shows a zoospore outside a root hair, and a zoospore about to penetrate a root hair is shown in fig. 2 of the same plate. Further evidence that the bodies inside the root hairs were zoospores that had penetrated from outside was provided by nuclear and cytological details which in all cases were identical.

According to LEDINGHAM (1934) the zoospores produced from the resting spores are biflagellate and heterocont. From haematoxylin stained, microtome sections, however, it was impossible to demonstrate the flagella ²⁾. In the present study, zoospores that had not yet penetrated the host cells, were rarely binucleate. It was concluded that infection normally occurs by the penetration of uninucleate zoospores arising from the resting spores.

The plasmodium

The development of the zoosporangial stage was first indicated by the presence of a multinucleate plasmodium in the young root hairs and epidermal cells

¹⁾ After penetration into the host cell, the zoospores, apparently having lost their flagella, develop from spherical bodies into young plasmodia. In the early stages of development the spherical bodies have been termed „amoebae” by some previous writers, but in this paper the term zoospore has been retained for this stage.

²⁾ Recent observations with the phase contrast microscope confirmed that the zoospores produced from the resting spores are biflagellate and heterocont.

(Plate I, fig. 7). The young plasmodia were situated against the walls of the root hairs and later, by continued growth, they occupied the entire root hair contents and may have extended from the root hair into the epidermal cell. In transverse sections of root hairs the plasmodia were seen to be more or less crescent shaped, and in the epidermal cells they lay against the outer tangential walls.

The plasmodium was reticulated by the presence of numerous very small vacuoles. The nuclei were characteristically „wheel-type”, the nucleolus being connected to the nuclear membrane by spoke-like, radiating threads. Observations of living root hairs in water mounts with the phase contrast microscope have confirmed this construction (Plate I, fig. 6, 8). The nuclei and nucleoli of very young plasmodia were usually considerably larger than those of older ones. At first the nuclei were often found lying in a row, but later they became scattered throughout the plasmodium. The plasmodial nuclei divided promitotically ¹⁾, the various „saturn”, „cruciform”, „double anchor” and telophase stages having been observed in the dividing nuclei (Plate I, figs. 9, 10, 11, 12 and 13). Since most plasmodial nuclei divided simultaneously only one division phase was usually found in a plasmodium.

The development of the zoosporangium

During further growth of the plasmodium the number of nuclei increased through promitotic divisions. At the end of the vegetative development of the plasmodium the zoosporangia were initiated through segmentation. Simultaneously the nuclei became indistinct and darkly stained granules appeared around them in the cytoplasm (Plate II, fig. 14). This phenomenon represents the akaryote ²⁾ stage. Observations with the phase contrast microscope on living roots in water mounts confirmed that in the akaryote stage of the plasmodium the nuclei were little or not differentiated (Plate II, fig. 15).

Following the akaryote stage, the young, zoosporangial nuclei divided mitotically (Plate II, figs. 16 and 17). Almost all the division figures found in prepared slides showed the metaphase and telophase stages. The number of nuclei and division figures in each segment varied, especially at the base of the root hairs and in the epidermal cells where the segments contained more nuclei and

¹⁾ Promitosis refers to a primitive type of nuclear division occurring in the Plasmodiophorales. The division is intranuclear and the nucleolus and the nuclear membrane persist during the division. During division, a ring of chromatin appears around the nucleolus („saturn” stage), the latter elongates („cruciform” stage) and the chromatin splits into two halves which move to the opposite ends of the nucleolus („double anchor” stage). With further elongation the nucleolus constricts and divides into two parts which, with the accompanying chromatin, become separated into daughter nuclei following the constriction of the nuclear membrane.

Promitosis may be regarded either as a primitive, direct type of division or as a special form of mitosis. The detailed examination and interpretation of nuclear figures clearly awaits further investigation, probably using methods of greater magnification and resolution than are provided by the limits of the optical microscope.

²⁾ The akaryote stage describes a condition of the nuclei at the end of the vegetative development of the plasmodium. The contents of the nuclei are little or not differentiated and strongly stainable granules appear in the cytoplasm surrounding the nuclei. The akaryote stage is soon followed by mitotic divisions of the nuclei. Present views on the nature of the akaryote stage are that either the chromatin is extruded from the nucleus into the surrounding cytoplasm leaving a cavity, or that the nucleus persists. In support of the latter view it is claimed that a fine chromatin reticulum and a faintly stainable nucleolus remain visible throughout the akaryote stage. The akaryote stage is of special interest since both hypotheses assume a nuclear fusion to occur.

division figures than in the middle and the tips of the root hairs. Counts were made of the number of nuclei and division figures present in numerous segments and the frequency per segment of nuclei in the akaryote, metaphase and telophase conditions is given in Table 1. The nuclei of superimposed segments, however, could not always be separately distinguished and furthermore the edge of the sections probably contained incomplete segments that had been cut through during microtoming, so that the values given probably contain large errors. The results indicated, however, that most of the segments contained two nuclei, though there were a number with 1, 3, 4 and 5.

TABLE 1. The number of nuclei in the young zoosporangia.
Het aantal kernen in de jonge zoösporangia.

Number of nuclei per segment <i>Aantal kernen per segment</i>	Frequency of the nuclei in the:			Total number of observations <i>Totaal aantal waarnemingen</i>
	akaryote stage	metaphase stage	telophase stage	
	<i>Frequentie van de kernen in het:</i>			
	<i>akaryotische stadium</i>	<i>metaphase stadium</i>	<i>telophase stadium</i>	
1	13	2	1	16
2	29	56	12	97
3	10	4	0	14
4	19	4	0	23
5	10	4	0	14
6	1	0	0	1
7	0	0	0	0
8	1	0	0	1

The next stages in the formation of the zoosporangia were the development into walls of the thin membranes delimiting the segments, and the formation of the zoospores (Plate II, fig. 20). Most of the zoosporangia contained in the root hairs, when formed side by side, appeared in longitudinal sections in square or rectangular forms (Plate II, fig. 21). The zoosporangia at the base of the root hairs and in the epidermal cells were commonly triangular, pentagonal or polygonal in shape. Zoosporangia that occurred singly and parts of zoosporangia that were not delimited by cell walls, were spherical in shape.

After the mitotic divisions the zoosporangial nuclei became almost unstainable, but later a ring of very small, stainable granules was found in the spherical membranes of the nuclei (Fig. I, *a*). The amount of stainable material in the ring increased and adjacent granules appeared to fuse (Fig. I, *b*). Eventually a sickle-shaped ring was formed by local thickening (Fig. I, *c*). The nuclear cavity gradually became filled with stainable material and eventually disappeared (Fig. I, *h*). The way in which the nuclei in the different stages of development were seen depended on their position in the sections (Fig. I, *d, e, f, g*; Plate II, fig. 18). The protoplasm of the zoosporangium finally separated into zoospores by the cleavage of the protoplasmic contents into uninucleate parts. The nuclei of the zoospores were at first disk-shaped and afterwards probably spherical (Fig. I, *i, j, k, l*). The zoospores themselves were spherical in shape and contained homogeneous cytoplasm (Plate II, fig. 19). On repeated occasions the fully-differentiated zoospores showed considerable movement prior to their liberation from the zoosporangia. It thus appeared that the fungus developed normally in plants in nutrient solutions, at least until the stage of zoospore formation.

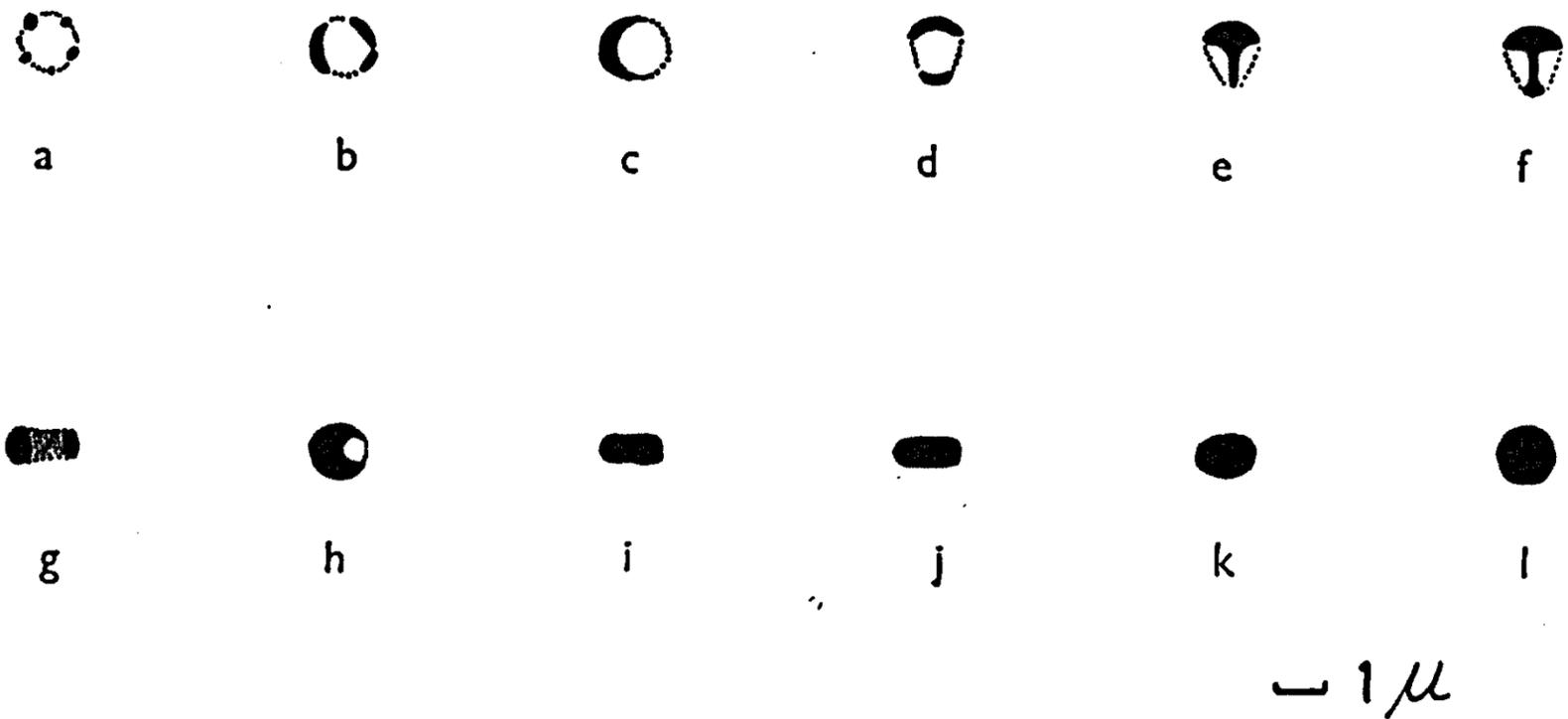


Fig. 1. Several stages in the development of the nuclei of zoospores in the zoosporangia. Explanation in the text.
Verskillende stadia in de ontwikkeling van de kernen van zoösporen in de zoösporangia. Verklaring in de tekst.

Counts of the numbers of zoospores in the zoosporangia are given in Table 2. These, like the counts made of zoosporangial nuclei, are subject to error. They show, however, that the zoosporangia generally contained either four, six or eight zoospores with a predominance of four. As previously described, a predominance of two nuclei per segment was found in the young zoosporangia

TABLE 2. The number of zoospores in the zoosporangia.
Het aantal zoösporen in de zoösporangia.

Number of zoospores per zoosporangium <i>Aantal zoösporen per zoösporangium</i>	Frequency <i>Frequentie</i>
1	4
2	4
3	7
4	53
5	6
6	16
7	7
8	25
9	0
10	7
11	2
12	4

(Table 1), and in many instances these nuclei were found to be dividing. The common occurrence of four zoospores per zoosporangium therefore seemed to indicate that only one mitotic division occurred in the development of the

zoospores. Owing to the relatively small number of division figures found and their extremely small size no further information on the nature of the zoosporangial divisions or on the possible occurrence of meiosis was obtained.

D. The zoospores

The process of zoospore emergence could best be studied from partially emptied zoosporangia in which the remaining individual zoospores could be distinguished. Before discharge, the zoospores can be seen moving within the zoosporangia. An opening, common to both the zoosporangial and host cell walls, provided an exit through which the zoospores were liberated. The openings frequently originated where the septum dividing two zoosporangia met the wall of the root hair, so that two zoosporangia discharged their zoospore contents through one opening (Plate II, fig. 21). The diameter of the openings was usually smaller than the diameter of the zoospores, which had to wriggle through the opening in succession. Although most of the zoospores were soon released, the discharge may continue for at least two hours.

Sometimes while freeing the long flagellum the zoospores exhibited an intense trembling motion. After their escape they either swam away instantly or remained for a short time on the surface of the root hair or a nearby cell and then swam away.

Freshly liberated zoospores swam at random. When swimming they were generally pyriform, though subject to changes in shape. As found by LEDINGHAM (1935), the zoospores were biflagellate and heterocont. The zoospore swam with the short flagellum in front and the long flagellum trailing behind. Occasionally when swimming the zoospore trembled violently. Eventually it slowed down, became stationary and assumed a spherical shape. The long flagellum may undulate for some time after it has become motionless. In the protoplasm of the zoospore, in which the nucleus and some vacuoles and granules may be distinguished, there was a continuous circulation.

Attempts were made to prolong the period of zoospore activity by liberation in twice distilled water, in water containing a high proportion of dissolved oxygen, or in the nutrient solution of VON KOPETZ & STEINECK. None of these methods gave periods of activity of more than 20–30 minutes. No evidence was obtained that zoospore activity had been influenced either adversely by the illumination to which the zoospores had been subjected under the phase contrast microscope, or favourably by working at relatively low room temperatures.

The stained zoospores were spherical in shape, biflagellate, heterocont and measured $4.60 \pm 0.08 \mu$ in diameter (Plate III, fig. 23). The average lengths of the long and short flagella were 13.70 ± 0.20 and $4.35 \pm 0.13 \mu$ respectively. The flagella were generally inserted close together. The zoospores usually possessed a spherical nucleus staining deeply with gentian violet (Plate III, fig. 24). A nucleolus could not be distinguished. In some preparations the nucleus appeared to be composed of several ellipsoid elements, arranged in a disk-shaped fashion.

A rhizoplast extended from the base of the flagellum to the nucleus (Plate III, fig. 26) and was readily distinguishable with the phase contrast microscope. Each rhizoplast, at the end nearest to the flagellum, contained a granular body, the blepharoplast (Plate III, fig. 25). The cytoplasm of the zoospore was finely grained and contained a number of small vacuoles. As first described by LEDINGHAM zoospores with two long and two short flagella have been found

(Plate III, fig. 27). The four flagella were inserted close to one another. Direct observations have shown that the quadriflagellate zoospores were the result of the fusion of two biflagellate zoospores. Two zoospores were observed to approach each other and become attached, and after an intense trembling motion, fusion of the pair occurred. The fused zoospores were momentarily constricted, but finally assumed a spherical shape (Plate III, fig. 31). In suspensions of zoospores the frequency of quadriflagellate forms increased, within limits, with increase in the swimming time of the zoospores.

Long continued observation showed that the nuclei of fused zoospores were subject to considerable changes in shape. However, the images obtained with the phase contrast microscope were too indistinct to permit a satisfactory interpretation of what was occurring. From cytological studies no evidence of karyogamy in the fused zoospores was obtained, for the two nuclei were generally found separated (Plate III, fig. 28). Occasional cases of apparent nuclear fusion were attributed to super-imposition of the separate nuclei (Plate III, fig. 29).

Zoospores have often been observed swimming around in pairs, at a short distance from one another. Despite the quick movements and the sudden changes in the direction of swimming, the distance between the paired zoospores remained constant. This phenomenon could be explained only by assuming a connection between the two zoospores. Observations with the phase contrast microscope showed that a connection was formed by a tiny thread which passed from a protuberance on one zoospore into a similar protuberance on the other of the pair. In one instance a zoospore couple was observed to swim for approximately one hour when it eventually came to rest without fusing (Plate III, fig. 30). It is possible that unfavourable circumstances may have accounted for the absence of a fusion. When in close proximity, zoospores that have come to a temporary standstill often touched each other with a protuberance. Intermittently this protuberance was withdrawn, only to appear elsewhere on the surface of the zoospore. When swimming zoospores approached each other, they were often affected by an intense trembling motion.

Compound zoospores each possessing three short and three long flagella have been found with the phase contrast microscope and also in permanent, stained preparations of zoospores (Plate III, figs. 32 and 33). The zoospores have been observed to result from the fusion of quadriflagellate and biflagellate zoospores (Plate III, fig. 34). After fusion, the hexaflagellate zoospores are subject to considerable changes in shape. The nuclei became very active, moving continuously throughout the cytoplasm. Sometimes the nuclei appeared to withdraw as far as possible from one another, the zoospore then assuming the shape of a triangle. The movements gradually slowed down and eventually the nuclei came to lie close together, though still separated.

When first formed, hexaflagellate zoospores swam actively, but eventually they became less active and finally came to rest. The protoplasm of stationary zoospores continued to circulate. Some of the granular inclusions in the protoplasm of the zoospores appeared to disappear and eventually the remaining inclusions were deposited around the periphery of the zoospores so that the protoplasm of the zoospores gradually became more hyaline. Whereas quadriflagellate zoospores were frequently found, hexaflagellate zoospores were found only occasionally in water mounts of living zoospores and in permanent stained zoospore preparations.

E. Discussion

Zoosporangial stages have been described in the life history of seven genera of the *Plasmodiophorales*.

From their work on *Plasmodiophora brassicae* WORON., COOK & SCHWARTZ (1930) considered that the germinating resting spores liberated uninucleate zoospores which infected root hairs of susceptible hosts, without any previous fusion. They claimed that after penetration the zoospore moved down the root hair, underwent nuclear division and developed into a small plasmodium, which, after cleaving into uninucleate segments, developed into zoosporangia. The nuclei of the young zoosporangia divide until there are four or six nuclei and these, with some surrounding protoplasm, develop into zoospores. Following their discharge, they suggested that the zoospores moved into the epidermal and cortical cells of the roots, where they fused in pairs, the zygotes formed giving rise to the further development of the fungus. AYERS (1944), was unable to confirm the discharge of the zoospores within the root hairs, while SAMUEL & GARRETT (1945) observed that whereas most of the zoospores escaped into the surrounding water, some were often found swimming within the cavity of the root hairs. COOK & SCHWARTZ did not fully describe the nuclear division of the plasmodium prior to zoospore formation. They considered that after cleavage of the plasmodium into segments, mitotic divisions of the nuclei occurred. From their description of the development of four or eight zoospores from the uninucleate segments it appears that several mitotic divisions occurred.

According to COOK (1926, 1928, 1933), in *Ligniera junci* (SCHWARTZ) MAIRE & TISON, the plasmodium develops from a uninucleate zoospore which enters the host through the root hairs or epidermal cells. The plasmodial nuclei divide promitotically and, after the akaryote stage, the zoosporangia develop from uninucleate segments. From his description of zoosporangia containing eight zoospores, two and sometimes three mitotic divisions must have occurred. As the number of zoospores produced in a zoosporangium is variable, it would appear that the original four nuclei may divide again independently of one another. In no instance more than eight zoospores have been found in the zoosporangium. Based on differences in size of the spindle and the metaphase plate it was concluded that the first division in the zoosporangium is heterotypic.

Polymyxa graminis, described by LEDINGHAM (1939), has no promitotic divisions of the nuclei throughout the whole period of growth of the zoosporangia. All divisions were of the ordinary mitotic type and even in the so-called resting nucleus no conspicuous nucleolus was present.

In *Octomyxa achlyae*, described by COUCH, LEITNER & WHIFFEN (1939), however, the nuclear divisions of the vegetative plasmodium and the zoosporangium were promitotic and mitotic respectively. Similar observations were made by GOLDIE-SMITH (1951) on *Sorodiscus cokeri* GOLDIE-SMITH.

The results of the present study are in general agreement with those of previous workers, obtained with other genera. The zoosporangia of *Spongospora subterranea*, however, may develop from multinucleate as well as from uninucleate segments, especially zoosporangia in the bases of root hairs and in epidermal cells. The promitotic divisions in the sporangial plasmodium are similar

to the descriptions of nuclear divisions described in *Ligniera junci*, *Octomyxa achlyae* and *Sorodiscus cokeri*.

The observations on the akaryote stage of *Spongospora subterranea* in the present study were similar to those described by COOK for *Ligniera junci*.

It would thus appear that the occurrence of mitotic divisions preceding the development of zoospores may be characteristic of all genera of the *Plasmodiophorales*. The exact nature of these divisions, however, has still to be explained, especially in relation to the occurrence of a meiosis. The present study of *Spongospora subterranea* has not revealed any indications of more than one mitotic division before the formation of the zoospores. LEDINGHAM does not mention the number of successive mitotic divisions in *Polymyxa graminis* preceding the formation of the zoospores. This number must be considerable as the zoosporangium begins its development as a uninucleate thallus and all the divisions are mitotic. COOK & SCHWARTZ and COOK indicate that several mitotic divisions occur in *Plasmodiophora brassicae* and *Ligniera junci*. In *L. junci* the first division is thought to be heterotypic although as previously stated this conclusion is based on differences in the sizes of the division figures and not on chromosome counts. It seems likely that the detailed examination and interpretation of nuclear figures must await further investigation, probably using methods of greater magnification and resolution than have hitherto been provided by the optical microscope. Moreover, the occurrence of a meiosis implies a previous karyogamy, which has yet to be confirmed.

The zoospores from the zoosporangia of the *Plasmodiophorales* were at first thought to be unflagellate: COOK described them as such for *Ligniera junci* and COOK & SCHWARTZ noted the presence of one flagellum in the zoospores of *Plasmodiophora brassicae*. LEDINGHAM first found that the zoospores of *Spongospora subterranea* and *Polymyxa graminis* were biflagellate and heterocont, and later similar zoospores were also found by COUCH, LEITNER & WHIFFEN and by AYERS in *Octomyxa achlyae* and *Plasmodiophora brassicae* respectively. The present study has confirmed the biflagellate and heterocont character of the zoospores of *Spongospora subterranea*.

The first indication of a fusion of zoospores in pairs came from the observations of COOK & SCHWARTZ on *Plasmodiophora brassicae*. They found instances of two zoospores lying near each other in the epidermal and cortical cells of the roots and also bodies with two nuclei. They concluded that a fusion stage in the life cycle of *P. brassicae* occurred. COOK (1926) was at first of the opinion that no fusion of zoospores of *Ligniera junci* occurred between the time of their liberation from the zoosporangia and infection of the new host, but later however, (1933) concluded that a fusion did occur. COUCH, LEITNER & WHIFFEN found double specimen among the biflagellate, heterocont zoospores of *Octomyxa achlyae*, which they attributed to an incomplete cleavage during the formation of the zoospores. Fusion of the zoospores was not observed, though such a fusion was thought to occur. In *Spongospora subterranea* and *Polymyxa graminis*, LEDINGHAM also found zoospores with a double number of flagella, of which two were long and two were short. As he did not observe a fusion, it remained in doubt whether these double zoospores were due to an incomplete cleavage or were the result of a fusion.

The present study has confirmed the findings of LEDINGHAM and shown that the double zoospores were the result of a fusion of two single zoospores. It

has been demonstrated, moreover, that not only two but also three zoospores may sometimes fuse.

At first it was thought that the fusion of the zoospores in pairs initiated a sexual stage in the life history of the fungus. However, this could not be proved because karyogamy was not observed in the fused zoospores. Assuming, however, that a zygote may be formed from the fusion of two zoospores followed by karyogamy, the fate of the product of karyogamy occurring in zoospores formed from two fusions (hexaflagellate zoospores) is difficult to explain. It should be remembered, however, that during the observations the zoospores were subjected to artificial conditions which may have unfavourably influenced the behaviour of the fungus. In the writer's opinion the fusion of zoospores can only be explained as an ordinary vegetative fusion that occurs as readily as anastomosis in some filamentous fungi. Whether or not the fusion is an essential part in the life history of the fungus is, however, as yet undetermined.

CHAPTER 2

THE INFECTION OF TUBERS, ROOTS AND STOLONS

A. Introduction

Spongospora subterranea produces Powdery Scab lesions on potato tubers and also wart-like growths on the roots and stolons. The manner in which tuber infection occurs has been previously investigated by several workers but in some respects the results of these studies are not in agreement.

According to KUNKEL (1915), the tuber tissue is invaded by the fungus in its plasmodium stage. A light brown spot at the centre of a translucent area marks the point where the plasmodium enters the skin of the tuber. Usually a considerable number of cells are killed at the point of infection. Once beneath the epidermis the fungus spreads out in all directions and forms a rather flat, disk-shaped mass, which separates the epidermis from the tissue beneath. In general, the potato cells in contact with the plasmodium are at once stimulated to abnormal growth and division and some of them may be killed as the plasmodium spreads throughout the healthy tissue. From the plasmodium, pseudopodia extend downwards between the cells of the healthy tissue and appear to push them apart. The plasmodium finally invades the cells through their softened walls and the cells of the infected tissue are stimulated to abnormal growth and division.

In the plasmodium described by KUNKEL, numerous globular bodies were found. They varied in size and stained very deeply with gentian violet and often appeared to be almost black. KUNKEL was unable to discover the origin of these bodies and was unable to observe details of their structure. They were always present and were very conspicuous in the infecting plasmodia and were carried with them into the potato cells. He concluded that they may be „encysted amoebae” that had been engulfed by the plasmodium. Nuclei were observed by KUNKEL in certain portions of the plasmodia, but they were poorly fixed and difficult to stain.

According to WILD (1929), the potato tubers are infected by plasmodia which invade the tubers through the lenticels as well as through wounds. OSBORN (1911) did not observe tuber infection and was unsuccessful in infection experiments. The earliest stage of infection observed by OSBORN was a single, uninucleate amoeba in a young potato cell near an eye. The earliest stage of infection found by COOK (1933) was an amoeba in the host tissue. PIARD-DOUCHEZ (1949) records the earliest stage of the infection of the roots as an invasion of the cells of the cortex by amoebae which rapidly fuse to form plasmodia. Tuber infection is thought to occur in a similar way.

The occurrence of numerous, deeply stained globular bodies in the plasmodium, the occurrence of characteristic nuclei in certain portions only of the plasmodium and the difficulty experienced in staining nuclei, provides evidence in the writer's opinion, that KUNKEL may have occasionally misidentified the plasmodia of *S. subterranea*. The identification as plasmodia of structures other

than those of the fungus is also possible in WILD's description of the fungus which contains no clear description of the plasmodium with the characteristic nuclei.

The descriptions of the later development of the infection also contain contradictory reports. According to KUNKEL shortly after the cells become infected they grow very rapidly and giant cells 5-10 times as large as normal sized cells become visible. The infected cells elongate and most of their growth is radially outwards. The giant cells finally cleave into smaller cells containing usually no more than 5 or 6 smaller cells. There were no indications that a plasmodium can pass from a growing infected cell into a healthy one. OSBORN (1911) and HORNE (1930) are likewise of the opinion that the amoebae cannot penetrate from an infected cell into a healthy one the parasite being passively distributed through the division of the host cells. According to COOK (1933) and PIARD-DOUCHEZ (1949), however, the amoebae may actively penetrate from one cell of the host tissue into another.

The later development of the fungus within the host has also been investigated by previous workers with fundamentally similar results. Resulting from the numerous host cell divisions following cell infection, wart-like excrescences develop on the surface of tubers, roots and stolons. The growth of the warts on the tubers is limited by the formation of a wound periderm in the tissue just beneath the site of infection. The wart tissue on the tubers gradually decays, leaving a shallow, crater-like depression filled with a fine, powdery mass of spore balls surrounded by the torn edges of the burst periderm. Though the development of warts on the roots and stolons does not appear to be checked by a wound periderm, these warts also decay and liberate their spore balls contents into the surrounding soil.

Differences of opinion occur between previous workers in the development of the fungus after infection. According to KUNKEL, the infection of developing potato tubers is by a plasmodium, which invades the tissue and infects a large number of cells at each site of infection. OSBORN, however, observed that the amoebae found in the earliest stages of infection continued to divide to produce multinucleate amoebae and, immediately before the akaryote stage, these amoebae coalesced to form plasmodia. The presence only of uninucleate amoebae in the youngest developed warts was also observed by HORNE. He provided further evidence of a multiplication of the amoebae after infection and also considered the implications of a mass infection. He also observed that plurinucleate as well as uninucleate amoebae were present in the majority of the host cells. In the cells of considerably older warts a preponderance of plasmodia was also observed but HORNE was unable to establish by direct observation whether these plasmodia originated from the fusion of amoebae, although he was of this opinion. According to COOK, the amoebae increase in size and simultaneously undergo nuclear division. After the plurinucleate amoebae have produced 6-8 nuclei, schizonts are cut off. COOK did not observe the development of plasmodia from the fusion of amoebae. PIARD-DOUCHEZ observed that the amoebae that cause infection rapidly fuse to form plasmodia.

The cytology of the fungus in the cells of the warts is one of the best studied parts of the life history of the fungus. The results of these investigations, however, differ in some respects. Most workers agree that the nuclei in the amoebae and in the young plasmodia divide promitotically. HORNE assumed a nuclear

fusion in the akaryote stage at the end of the vegetative development of the plasmodium. According to OSBORN the fusion occurs just after the akaryote stage. COOK, on the contrary, found no evidence of a fusion at this stage in the development of the fungus. The akaryote stage is soon followed by mitotic divisions of the nuclei and the plasmodium matures into a spore ball composed of resting spores. There is little agreement on the nature and the number of mitotic divisions following the akaryote stage. According to OSBORN, two divisions of the nuclei follow each other rapidly. The second division differed from the first division in possessing nuclei with shorter spindles and more sharply defined fibres. In the second division 8 chromosomes were observed. KUNKEL also observed the occurrence of mitotic divisions preceding the formation of the resting spores, but was unable to confirm the occurrence of two successive divisions. HORNE described two different divisions; a heterotypical and a homotypical division occurring in succession. These meiotic divisions were sometimes followed by a third mitosis, which immediately preceded spore formation. It is possible that the second division described by OSBORN is the same as the first division described by HORNE for, according to the latter, the diploid number of the chromosomes is 8. COOK also distinguished a heterotypical and a homotypical division with respectively 8 and 4 chromosomes but he obtained no evidence of a third division.

As mentioned in the general introduction, *S. subterranea* may continue to develop within the tuber after lifting. One of the most serious aspects of Powdery Scab in the U.S.A., according to KUNKEL, is the development of dry rot that develops from the sori during autumn and winter in stored tubers. Sections of the shrunken areas around old sori showed plasmodia pushing into the cells through the softened cell walls. The invaded cells were quickly killed. The plasmodium appeared to pass from cell to cell as they became successively destroyed. KUNKEL attributed the occurrence of plasmodia to the germination of the resting spores present in the original infected spot. Very young plasmodia were seen in sections of some sori that were just beginning to show dry rot, while many of the spore balls in the bases of old sori showed germination. KUNKEL compared the effect of the plasmodium on the growing cells of the young tuber with the effect of the plasmodium on the mature cells in the tissue around the old sori of lifted tubers. He found that cells of young tubers were not killed, but were stimulated to increased growth and division to produce a raised sorus. The cells around old sori on lifted tubers however, were quickly killed and discoloured areas on the skin resulted. MELHUS, ROSENBAUM & SCHULTZ (1916) observed the influence of external conditions on the development of the fungus on stored tubers. They found that if a tuber showing incipient infection was detached from the parent plant and placed in a moist chamber further development of the infected area became seriously checked. They found also that if the stems of potato plants were cut off at soil level, the early stages of infection on the tubers failed to give rise to pustules though the discoloured areas may increase in size and the epidermis may become slightly raised, suggesting that the plasmodium continued to develop vegetatively without the production of spore balls. It was further noticed that if the plant continued growth after infection had occurred and external conditions were favourable for the development of the fungus the sori increased in depth and diameter.

The present study is an attempt to enquire further into the earliest stages of

infection and the development of the fungus after infection. A study was made of the number of mitotic divisions which occur prior to spore formation and the spread of infection from the Powdery Scab lesions in lifted tubers.

B. Materials and methods

Infected tubers, roots and stolons have been either collected in the field or obtained from plants artificially inoculated in pots. For cytological work small pieces of tuber showing infection in various stages of development were cut out. Pieces of roots and stolons bearing warts and small lengths of rootlets showing symptoms as local thickenings were also used.

The material was fixed in Flemming's killings solution (RAWLINS, 1931), embedded in paraffin-wax according to either the alcohol-xylol or the tertiary butyl alcohol methods of JOHANSEN (1940). Sections were made with a rotary microtome at thicknesses varying from 3–12 μ . Most of the sections were stained with Heidenhain's haematoxylin (JOHANSEN, 1940). In order to study some sections with the phase contrast microscope, some sections were unstained and were mounted in Canada balsam after the removal of the paraffin-wax with xylol.

C. The development of infection

The earliest stages in the infection of the tubers as seen with a $\times 10$ hand-lens were very small, circular spots, 1–2 mm. in diameter. The infected spots were characterized by a purplish-brown discolouration of the tuber tissue beneath the skin. In most instances the discolouration began from a lenticel (Plate IV, fig. 35). From microscopical observations it appeared that the purplish-brown discolourations were due to necrosis of numerous cells of the inner layers of the skin and the outer layers of the cortex. The walls of the necrotic cells were somewhat swollen and darkly stained, while the contents were coarsely granular and only lightly stained. Many of the cells adjacent to the necrotic cells contained plasmodia and in some instances amoebae, confirming the presence of *S. subterranea*. Some cells at a distance of approximately 20 cell-widths from the edge of the lenticel were found to contain plasmodia (Plate IV, fig. 36, 37), indicating that the fungus had moved beneath the skin and moved sideways from the lenticel. The actual penetration of cells by the fungus was however, not observed. The next stage in the development of the disease was a slight raising of the surface of the tuber at the site of the infection accompanied by an extension of the purplish-brown tissue discolouration into the healthy tissue. Microscopical examination showed that the necrotic cells occurred in deeper layers of the cortex and that they spread by means of strands of necrotic cells which penetrated radially from the edge of the subperidermal layers (Plate IV, fig. 38, 39). Amoebae and plasmodia were found only in the cells immediately adjacent to the necrotic strands (Plate V, fig. 44, 46, 47). These infected cells were often much enlarged and their nuclei showed signs of deformation while others, including some apparently healthy cells, were in a state of active division. The raising of an infected spot on the surface of the tuber therefore, appears to be due to cell enlargement and also to a lesser extent, cell division.

The presence of cells containing the fungus in an active state in the margins of the necrotic strands suggested that the invasion of the cortex occurred through

the extension of these strands. Numerous deeply staining bodies were present in the contents of these cells (Plate IV, fig. 40, 41). The contents of these bodies were frequently kidney-shaped lying against a thin wall. The bodies were found only in the necrotic strands of the early stages of infection. In the tips of the necrotic strands *Spongospora* amoebae, deeply staining bodies and forms intermediate between these were found (Plate V, fig. 42, 43). In the present study it was not possible to determine the exact nature of this relationship, nor was it possible to determine the origin of deeply staining bodies but two possibilities occur.

(a) It is possible that amoebae develop from these bodies which represent the fungus in its infection stage in which case the presence of bodies without deeply staining contents could be explained. The accumulation of the bodies in the corners of the cells suggests a possible migration of the bodies between cells, though evidence of a penetration through the cell-wall was not observed. The thickened cell-walls and the necrotic tissue, however, rendered such observations difficult. Despite the occurrence of numerous bodies no evidence was obtained of their ability to multiply.

(b) If the amoebae represent the fungus in its earliest stage of infection the bodies may be the remains of amoebae in necrotic cells. This possibility would explain the presence of amoebae only in the tips of the necrotic strands.

Eventually necrosis of the infected tissue ceases though further cortical cell infection without cell necrosis may occur.

With later development of the fungus, the infected spot on the surface of the tubers may become further raised. The tissue adjacent to the purplish-brown discoloured tissue becomes somewhat watersoaked. Further extension of the discoloured tissue may occur but the depth at which the necrotic strands extended into the cortex varied; some necrotic strands extending to the outer layers of the cortex only, while other strands were found in deeper layers.

When infected cells are not killed they often become more or less enlarged and sometimes they develop into „giant cells” (Plate VII, fig. 57), the nuclei of which are often deformed. Other infected cells may divide to produce cells similar in dimensions to normal cortical cells. The formation of the new tissues causes the skin of the tuber to burst and the outer tissues of the infected zone may protrude above the surface of the tuber in a „cauliflower” form (Plate VII, fig. 57, 58). The divisions produced by infection are mostly radially outwards from the base of the infection. Further development of the fungus continues and in the newly infected cells plasmodia and amoebae occur. During cell division the fungus is passively distributed over the newly formed tissue (Plate VI, fig. 48, 49, 50). The nuclei of the amoebae and plasmodia divide promitotically (Plate V, fig. 45). Eventually, no further division of the host cells occurs, when the amoebae coalesce to form plasmodia. The plasmodial nuclei undergo an akaryote stage and afterwards the formation of the resting spores occurs with mitotic divisions of the nuclei (Plate VI, fig. 51, 52, 53, 54, 55). During these divisions the plasmodium segments by the production of thin membranes around the nuclei, and apparently by contraction of the segments, the plasmodium develops hollows.

Ultimately the plasmodium changed into a spongy aggregate consisting of numerous resting spores (Plate VI, fig. 56). The resting spores are approximately polygonal in section and hexagonal when closely packed (Plate VIII, fig. 64).

The spores are thick walled enclosing a spherical body within. The spherical body does not completely fill the resting spore and is destined to give rise to a zoospore. Usually the nucleus of the resting spore is difficult to distinguish but it was occasionally observed as a darkly stained spherical body in the cytoplasm. After the last mitotic division during the formation of the resting spores, the nuclei first appear to be stained only in the periphery. Later the contents of the nuclei appear to consist entirely of chromatin.

Except under excessively wet conditions, the development of the warts ceased when the skin of the tuber overlying the warts was ruptured. A wound periderm develops beneath the infected spot isolating the lesion from the healthy tissue beneath and finally only a small crater remains containing host cells and spore balls surrounded by the raised edges of the burst periderm (Plate VIII, fig. 63). Under excessively wet conditions however, the wound periderm may not develop and wart development may continue with the development of very large warts. Some warts may show both decay and continued development around the margins, probably resulting from imperfect periderm formation.

The early stages of infection of the roots as well as tubers resemble each other closely. Thus, with root infection the infected tissue undergoes necrosis, typical darkly-stained bodies are present in the necrotic cells and in the surrounding cells amoebae were present (Plate VII, fig. 59). Several cells containing amoebae were much enlarged and contained unusually shaped nuclei. The infected cells of the root also underwent active cell division accompanied by typical wart formation (Plate VII, fig. 60). The cells of these divisions are less radially orientated and more irregular in position than the cells developed in the warts on the tuber. Wart development on roots and stolons interferes considerably with the normal structure of the tissue (Plate VII, fig. 61). Thus, in transverse sections, young thin roots can only be distinguished by the primary wood vessels occurring in the middle of the diseased mass of wart tissue. Sometimes through disturbances of the cambium, vessels may develop in the wart tissue. Such development on older, thicker roots and stolons is restricted however to a sector only. At first the warts are milky-white in appearance but in contrast to wart development on the tubers, the development of the warts on the roots is not checked by the formation of a wound periderm. Apparently there are other factors preventing continued development of the warts on the roots and stolons. The plasmodia develop into spore balls (Plate VII, fig. 62), and finally the tissue of the wart is killed. The warts finally turn brown and decay and the spore balls are freed in the soil.

D. Nuclear divisions preceding resting spore formation

Only a few mitotic nuclear divisions were found in the plasmodia and information on the number of subsequent divisions prior to resting spore formation was obtained from only one slide made from a relatively large wart. Numerous plasmodia however with dividing nuclei were found and consecutive stages in the development of the plasmodia were found from the centre to the edge of the wart. In the centre of the wart the fungus occurred in the vegetative stage only and near the border the plasmodia had developed into spore balls.

Microscopical observations indicated the occurrence of two and probably three consecutive mitotic divisions of the plasmodial nuclei. The first two divisions were distinguished by the shape of the spindles and the size of the

metaphase plate. The spindle of the second division was thinner than the spindle of the first while the metaphase plate of the first division was larger than the metaphase plate of the second division.

An attempt was made to determine the number of successive nuclear divisions in the plasmodium by counts of the number of nuclei. Assuming that all the nuclei of a plasmodium divided simultaneously, the numbers of metaphase figures of the first and second divisions were compared. The overlapping of the spindles of some nuclei however, in sections with thicknesses of 10μ made exact counts of the number of division figures by direct observation difficult. For more accurate and rapid counting the following method was employed. Using a camera lucida fitted to the microscope the nuclei were marked on a sheet of paper beneath which was placed a second sheet of paper separated from the first by carbon paper. The metaphase plates of the division figures were represented with circles and dashes depending on whether the spindles were in a vertical or a horizontal position respectively. The nuclei were marked on the upper sheet of paper with a steel-ball pointed pen so that while the upper sheet remained unmarked the nuclei were noted on the second sheet of paper in carbon. In this way all the division figures in a plasmodium were marked out and counts of the circles and dashes gave the total number of division figures per section of the plasmodium. The authenticity of the method was tested by determining twice the number of division figures in two characteristic plasmodia. The results were respectively 60 and 62 and 60 and 59 division figures per plasmodium. The counts of nuclei in 20 plasmodia in which the nuclei were involved in the first division, and of nuclei in 20 plasmodia in which the nuclei were involved in the second division, gave 36.20 ± 2.67 and 74.35 ± 4.37 division figures per plasmodium respectively. These results agree with the theoretically expected ratio of 1:2 and confirm the existence of two successive divisions (Plate VI, fig. 51, 52, 53, 54).¹⁾

It has not been possible to compare the number of division figures of the first division with the number of nuclei in the plasmodia at the end of the vegetative stage. The number of prophase figures and metaphase figures of the first division have however been compared. The mean number of prophase figures per plasmodium was 39.15 ± 1.46 . Thus, the difference between this and the number for metaphase figures of 36.20 ± 2.67 is small enough to confirm that both stages are identical. The number of divisions preceding spore ball formation is however obscure. Unsuccessful attempts were made to count the number of resting spores in the sections of the spore balls in a way similar to that employed for the counts of plasmodial nuclei. Thus, the estimated number of resting spores varied between 200 and 500 indicating a possible third division while a few plasmodia that were found with small telophase figures may also represent a third division. Only one plasmodium was found however with metaphase figures which may have represented a third nuclear division (Plate VI, fig. 55). In this instance the number of division figures was 137. These data approximate the expected value of 149, which is twice the number of nuclei at the second division.

¹⁾ The interval of reliability of the ratio is 0.40–0.59 on the 5% point. The theoretical value of 0.5 is within these limits and consequently the outcome is not in contradiction with the expected ratio. I am greatly indebted to Dr E. F. Drion of the „Afdeling bewerking waarnemingsuitkomsten van de Centrale Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek”, Den Haag, for advice and assistance on the statistical aspects of the work.

From this part of the investigations it is certain that there are two and probably three and perhaps even more consecutive mitotic nuclear divisions prior to resting spore formation.

E. The influence of the time of lifting on the development of tuber infection

The time of tuber lifting and storage conditions appear to exert an influence on the development of tuber infection. If the tubers are lifted at a time when the infected tissue has not burst through the periderm, the development of the disease may be different from that occurring under natural conditions. Abnormal development is characterized by a discoloured area of necrotic tissue beneath the skin, often accompanied by the formation of a ring of necrotic tissue in the healthy tissue surrounding the infection and sometimes a second ring surrounding the first ring may be formed. If tubers are lifted other developments may occur when the infected tissue has burst through the skin however. Under dry conditions the tissue of the wart decays as already described while under humid conditions, the infected tissue results in the development of somewhat larger warts and also secondary warts alongside the primary warts accompanied by little discolouration and necrosis of the tissue beneath the skin.

From microscopical observation it appears that the necrosis of the tissue around the original infected spot is due to a subperidermal spread of the infection (Plate VIII, fig. 66). The fungus does not however induce the infected cells to divide, while the fungus spreads from cell to cell in the subperidermal layers of the cortex. The infected cells have a shrunken and compressed appearance. In the periphery of the necrotic area the fungus can be distinguished as amoebae and plasmodia. The plasmodia develop somewhat rapidly into spore balls while simultaneously infected cells are killed resulting in a discolouration of the tissue. The spore balls are shaped in the form of the cells which contain them. With further storage streaks of necrotic tissue and sometimes cavities may be formed beneath infected spots (Plate VIII, fig. 65), though no signs of the presence of the fungus could be detected in such tissue. Whether such necrotic tissue is caused indirectly by the presence of the fungus on the surrounding tissue is not known.

Thus, when potatoes are lifted in early stages of infection, the fungus loses the ability to induce cell division in the infected cells although it may migrate into the outer layers of the cortex killing the cells invaded. If there is visible wart formation at the time of lifting however, the fungus develops further as under natural conditions.

F. Discussion

In the present study neither the actual method of infection was determined nor could KUNKEL'S observation on infection with plasmodia be confirmed. Thus if infection was caused by a plasmodium, it may be expected that the plasmodial nuclei would be visible, yet in only certain parts of the plasmodium was KUNKEL able to discern nuclei which were poorly fixed and rather difficult to stain.

The presence of the deeply stained bodies may be regarded as identical with those described by KUNKEL who regarded them as encysted amoebae that had probably been engulfed by the plasmodium. These bodies probably represent the fungus in its earliest stages of infection.

KUNKEL's opinion that the infected cells grow very rapidly and attain a size of 5 or 10 times as large as normal cells could not be confirmed in some respects. Thus at first many cells do enlarge and giant cells may be formed, but this host reaction did not always occur following infection. The number of cells produced from the division of infected cells was not found to be limited as stated by KUNKEL, but cell division was found to continue as long as circumstances permitted wart development.

According to PIARD-DOUCHEZ, following tuber invasion by amoebae, no external symptoms of infection developed until a fusion of the amoebae into plasmodia occurred. No evidence of the necessity of this fusion prior to external tuber symptom development was obtained in the present study.

These studies showed that there may be two phases in the development of the infection. The first phase is characterized by the penetration of the fungus resulting in necrosis of the invaded cells; followed by a second phase in which the infected cells are stimulated to continuous cell division resulting in the formation of warts. The transition between the two phases represents a phase in which the cells are not killed but show hypertrophy and deformation of the nuclei.

The studies of the development of the warts on the tubers, roots and stolons and the number of consecutive mitotic divisions of the nuclei before spore formation, are generally in agreement with those of previous work.

Studies of the influence of the time of lifting on the development of tuber infection were in agreement with KUNKEL's studies of the secondary infection of the tissue around the old sori except in the origin of the migrating plasmodium. According to KUNKEL, this plasmodium originates from the germination of the spore balls in the base of the old sori. In the present study the migrating plasmodia were considered to arise from the infected cells of the cortex, in which the fungus is still in a vegetative stage at the time of tuber lifting. The spread of the infection around the originally infected spot occurring during tuber storage moreover, was only found to occur if the skin covering the infected spot remained unbroken.

PART II

THE RELATIONSHIP OF ENVIRONMENTAL
FACTORS TO INFECTION

CHAPTER I

THE GERMINATION OF THE RESTING SPORES

A. Introduction

The germination of the resting spores has been described by KUNKEL (1915), COOK (1933), LEDINGHAM (1934) and PIARD-DOUCHEZ (1949). According to KUNKEL the walls of the spore ball sometimes disintegrated producing as many amoebae as spore ball cells. Generally the amoebae were observed to escape through openings in the walls of the individual spores. The spore ball after germination remained almost intact. Numerous spore balls composed entirely of empty spores were seen in some preparations. Each spore contained one nucleus and produced on germination a single, uninucleate amoeba. The amoebae were small, but under favourable conditions they grew rapidly and divided. Amoebae moved by means of pseudopodia and no cilia were observed. COOK (1933) stated that the resting spore zoospores were unflagellate, each zoospore possessing a flagellum equal in length to that of the zoospore. The presence of a flagellum appeared to depend upon the amount of liquid present at the time of zoospore liberation. Thus in dry media the zoospores immediately became amoeboid. COOK described zoospores fusing in pairs and if present at the time of fusion the flagellae were withdrawn and the two zoospores conjugated by their anterior ends. After fusion a nuclear fusion occurred and occasionally the newly formed zygotes were flagellate. LEDINGHAM (1934) described the zoospores from germinated resting spores as biflagellate, each zoospore possessing one long and one short flagellum. According to PIARD-DOUCHEZ (1949): „Nous avons vu avec netteté l'ouverture des parois de sporanges, mettant en liberté des amibes minuscules qui grossissent rapidement; les tailles de ces amibes sont très variables; les noyaux sont bien visible ainsi que le membrane nucléaire et les mailles du cytoplasme.” Her publication contains no reference to the occurrence of flagella.

Difficulties of interpretation of the method and products of resting spore germination may be expected since the spore balls consist of aggregates of very small resting spores, which are difficult to observe microscopically. The walls of the resting spores are strongly refractive and often darkly coloured. The presence of holes between the aggregates of resting spores moreover may harbour flagellate organisms other than *S. subterranea* leading to misidentifications.

KUNKEL described a method of obtaining abundant spore ball germination. Mature spore balls obtained from sori were dusted over the surface of lima-bean or potato agar in petri dishes, just prior to the gelling of the media. Observations

showed spore ball germination after a few days. COOK germinated resting spores using the method of KUNKEL, except that after 1–2 days a small quantity of sterile potato extract was added to the agar surface. LEDINGHAM obtained great numbers of zoospores by germinating resting spores in dilute inorganic nutrient solutions, though the spores were previously moistened, frozen and dried on several occasions. PIARD-DOUCHEZ used water agar instead of nutrient agar but otherwise employed the method as described by KUNKEL.

Attempts were made to germinate resting spores by the method described by LEDINGHAM and on several occasions large numbers of zoospores were obtained. When examined after killing and staining the zoospores agreed with the zoospores of *S. subterranea* described by LEDINGHAM. Unfortunately the method gave no information on the method of germination or the presence of emptied resting spores and within a few days the solutions were contaminated by numerous secondary micro-organisms.

The methods of germination employed by KUNKEL and COOK were successfully repeated but these methods also gave no information on the actual method of germination.

Root-hair infection in nutrient solutions provided evidence of the germination of spore balls and using this method studies were made of the conditions necessary for germination.

B. Materials and methods

SAMUEL & GARRETT (1945) used the root-hair infection of cabbage seedlings for estimating the activity of the zoospores of *Plasmodiophora brassicae* in the soil. In their study the degree of infection was determined by counting the number of infected root hairs along a standard length of the root. It was found however in the present study that the variation in root-hair infection was large and the method could not be employed. The amount of infection was however rated microscopically as heavy, moderate, slight and sporadic. This rating was based on the number of infected root hairs on 1 cm. lengths of root mounted in water on glass slides. Each rating was based on the examination of six lengths of root. The composition of the nutrient solution and inoculation techniques used have been previously described in Part I, chapter 1. For the examination of zoospores, a phase contrast microscope was sometimes used.

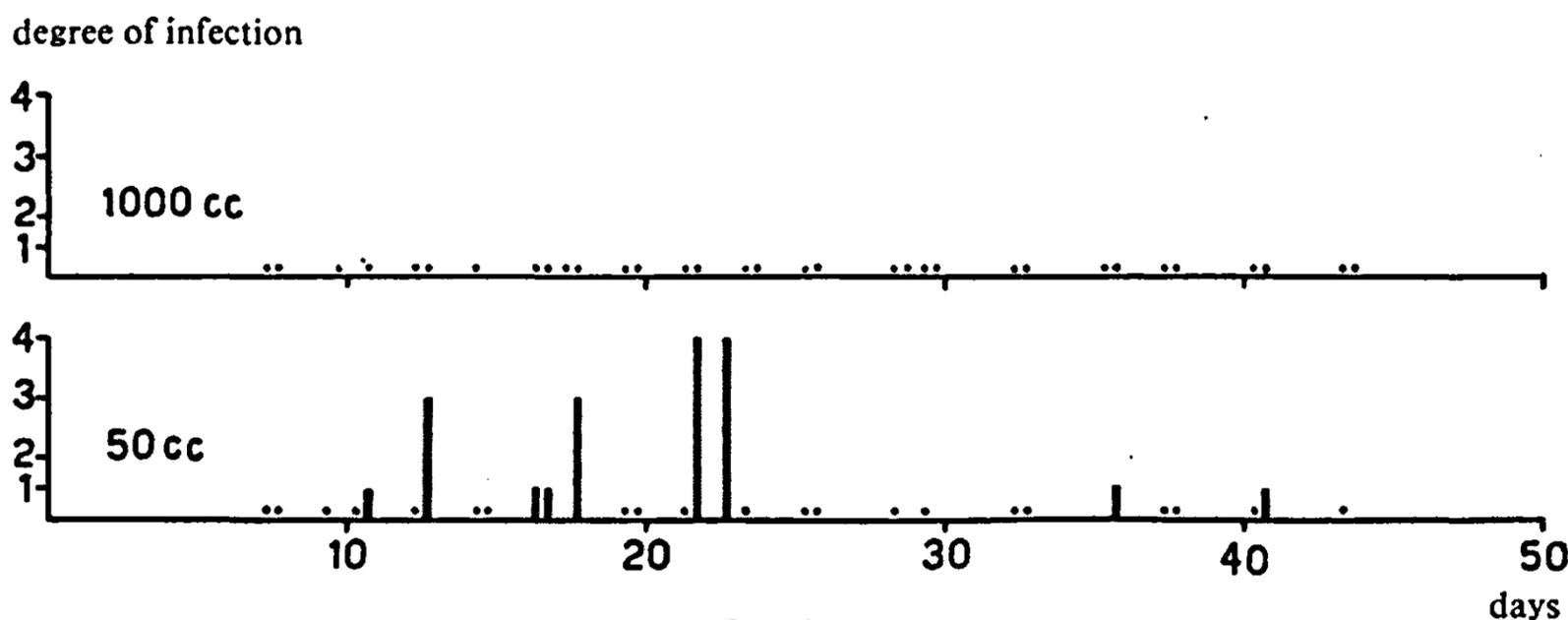
C. Root-hair infection of tomato seedlings in nutrient solutions

Preliminary infection experiments in nutrient solutions were made with potato and tomato plants in 650 cc. jam jars. To exclude light the jars were painted on the outside. The plants were fixed with cotton wool into the holes of the jar covers from which the plants were suspended. When the root systems of the plants had developed sufficiently spore balls were added to the solution. The roots were periodically examined for root-hair infection by microscopical examination. In potato and tomato plants infection was obtained 39 and 45 days respectively after inoculation.

The experiment was repeated with tomato plants only, using 50 cc. glass jars and the plants were artificially illuminated. Inoculations were made in the manner previously described and infection was obtained after 8–12 days.

In a further experiment jars of 1000 and 50 cc. capacity were used containing

nutrient solutions. Tomato seedlings previously grown in garden soil were grown in the jars until they had reached the 2-4 leaf stage when inoculations were made with about 3 mg. spore balls. Two jars of each size were used. To prevent the spore balls from floating on the surface of the nutrient solutions when added they were previously soaked in a small quantity of water. The experiment was done in a glass-house with a constant temperature of 18° C. The tomato plants were replaced at successive and equal intervals of time from the beginning of the experiment. By successive examination of the root-hair infection which resulted, a measure of the germination of the resting spores was obtained. Examination of root-hair infection commenced eight days after inoculation. No infection occurred in the plants growing in the 1000 cc. jars although successive examinations were made for 44 days after inoculation. In one 50 cc. jar, however, infection occurred after 11 days and continued for a further 12 days. In this jar infection also occurred sporadically after 36 and 41 days. In the duplicate 50 cc. jar infection was obtained on the 17th day after inoculation. The results of the experiment are given in graph 1 where the lines of varying length indicate the degree of infection obtained and the dots represent absence of infection.



Graph 1

The influence of the quantity of nutrient solution on the occurrence of root-hair infection.

• no	infection
1 sporadic	„
2 slight	„
3 moderate	„
4 heavy	„

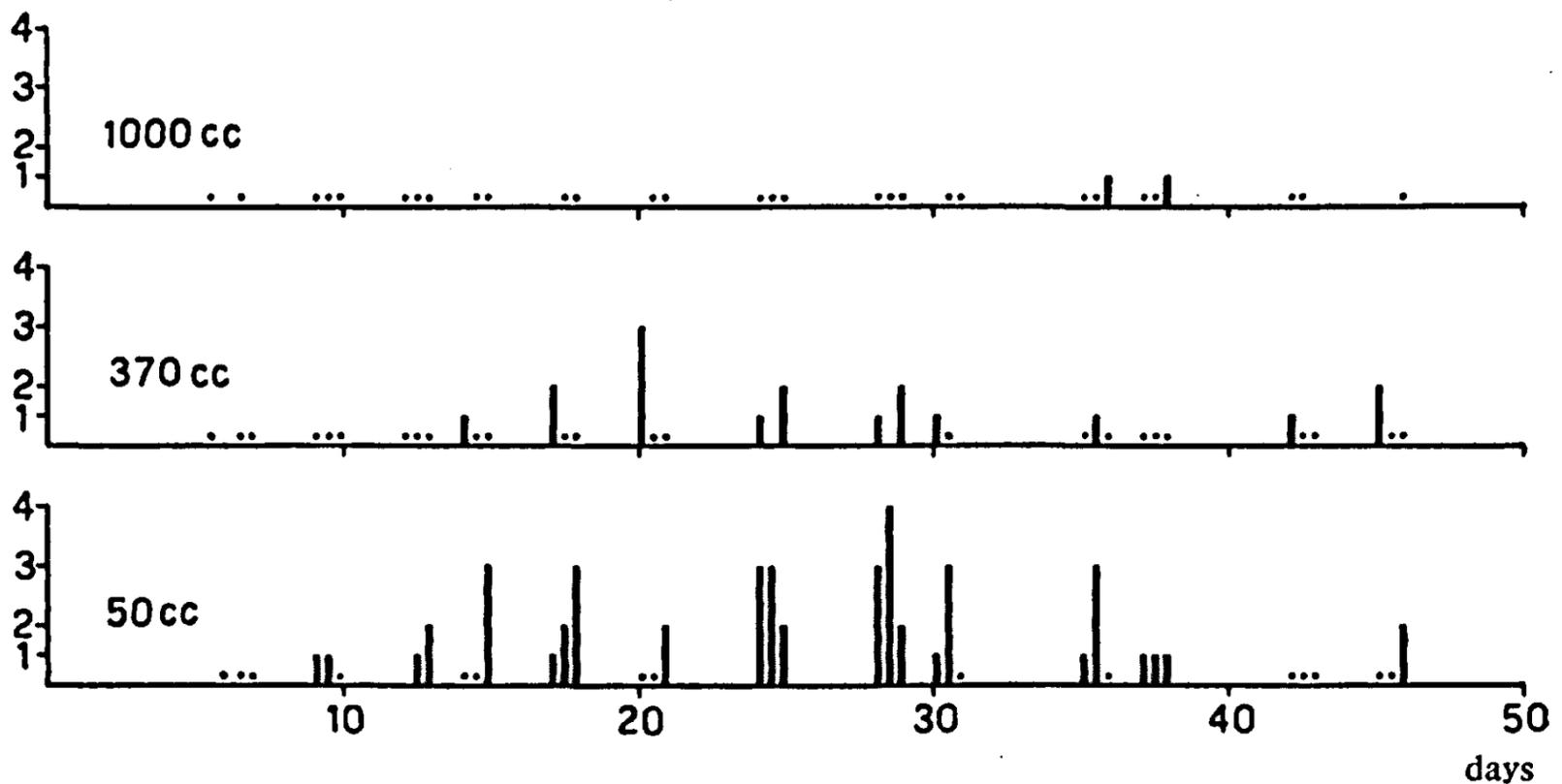
De invloed van de hoeveelheid vloeistof op de aantasting van de wortelharen bij watercultures.

• geen	aantasting
1 sporadische	„
2 geringe	„
3 matige	„
4 sterke	„

The experiment was repeated using 50, 370 and 1000 cc. jars using three jars of each size. The roots of two plants in the 50 cc. jars became infected 10 days after inoculation and the roots of the third plant 13 days after inoculation. In all three plants infection continued until the 38th day after inoculation. In one jar slight infection also occurred after 46 days. Much less root-hair infection occurred in the 370 cc. jars. In one jar of 370 cc. infection occurred after 15 days and in the others after 25 and 36 days respectively. One jar of 1000 cc. showed sporadic infection after 36 days. These results are given in graph 2.

Up to this point in these experiments inoculations had been made with equal quantities of spore balls to each plant irrespective of the capacity of the jars. In

degree of infection



Graph 2

The influence of the quantity of nutrient solution on the occurrence of root-hair infection.

• no	infection
1 sporadic	„
2 slight	„
3 moderate	„
4 heavy	„

De invloed van de hoeveelheid vloeistof op de aantasting van de wortelharen bij watercultures.

• geen	aantasting
1 sporadische	„
2 geringe	„
3 matige	„
4 sterke	„

a final experiment the inoculum potential was adapted to the capacity of the jars. To the solutions in three 50 cc. and three 1000 cc. jars, 3 and 60 mg. spore balls respectively were added. Earlier, heavier and more frequent infection was obtained in the 50 cc. jars, thus confirming the results of the former experiments.

The possibility of root excretions influencing the germination of spore balls was tested by germinating spore balls on plates of water agar. Root exudates, obtained from the leachings of pots of sand in which potato plants had been grown, were poured on to the surface of the agar. The evidence of spore ball germination in these experiments, however, was no more convincing than in the experiments in which the methods of KUNKEL and COOK were employed.

In spite of this, since the results of the present study show that earlier and more frequent root-hair infection was obtained in plants growing in small quantities of solution, it is suggested that the presence of the host plant may influence the germination of resting spores.

D. Discussion

The ease with which it has been possible for previous workers (KUNKEL, COOK, LEDINGHAM and PIARD-DOUCHEZ) to germinate resting spores is difficult to understand for it is well known that under field conditions the resting spores may persist for many years. In the light of the results of the present study the persistence of the fungus in the soil may be explained by the supposition that the germination of the resting spores is determined by the presence of a host plant.

CHAPTER 2

THE EFFECT OF PRE-TREATMENT ON THE GERMINATION OF RESTING SPORES

A. Introduction

In previous inoculation experiments tomato plants were grown in garden soil containing the dried spore balls of *S. subterranea*. The spore balls were obtained by pulverizing and sifting the dry scrapings from the powdery scabs of diseased potato tubers. Better infection was obtained in plants growing in soil, freshly inoculated with the dry spore balls, than in soil/spore ball mixtures that had been stored under moist conditions. The results suggested that the germination of the spore balls could be affected by storage conditions.

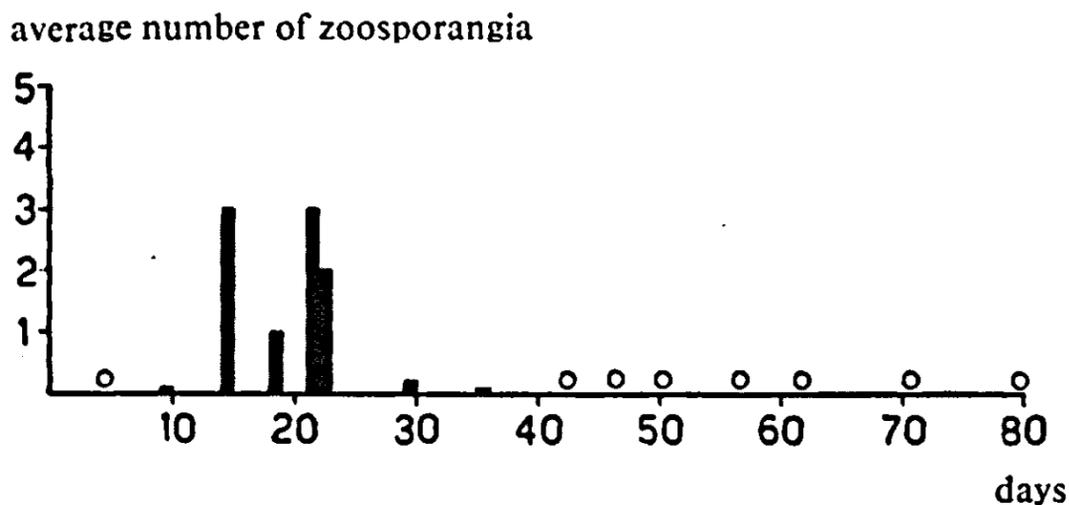
B. Materials and methods

The influence of pre-treatment on the germination of the resting spores was studied by root-hair infection of tomato plants, grown in heavily inoculated soil treated in various ways. Tomato plants were raised in seed-pans and as soon as the first pair of true leaves had developed they were transplanted into infected soil in 8 cm. pots. Each experiment consisted of three pots each containing five plants. After planting, the plants were waterlogged for a short time and placed in a glass-house at a constant temperature of 18 °C. Throughout the experiments the soil in the pots was kept wet by daily watering. The incidence of infection was measured by the method previously described in Part II, chapter 1. The plants to be examined were carefully removed from the pots and the root systems were carefully washed. Pieces of the roots bearing root hairs were cut into lengths of 1 cm. and mounted in water for examination. The removed plants were replaced by healthy plants propagated by the method previously described.

C. The effect of pre-treatment on the germination of resting spores

In a preliminary experiment garden soil heavily infected with resting spores was spread thinly and air-dried in the laboratory. Using tomato seedlings the germination of resting spores in the soil was tested by the determination of root-hair infection obtained, based on counts of the number of zoosporangia present in the root hairs. Roots bearing root hairs were mounted in water and examined microscopically. Parts of the roots bearing root hairs were brought into the centre of the optical field and the number of zoosporangia were counted along a length of root equal in length to the diameter of the optical field. Sixteen counts were made per plant and the mean number of zoosporangia per unit length of root is shown in graph 3 where infection is plotted against time. The individual root counts of zoosporangia varied considerably and the results can only be taken as general indication of the course of root-hair infection. Zoosporangia were found in the root hairs from 10–37 days after the beginning of the experiment and although the experiment was continued for 80 days no

further zoosporangia were found. The experiment was repeated when root-hair infection occurred from 19–35 days; the experiment being discontinued after 61 days.

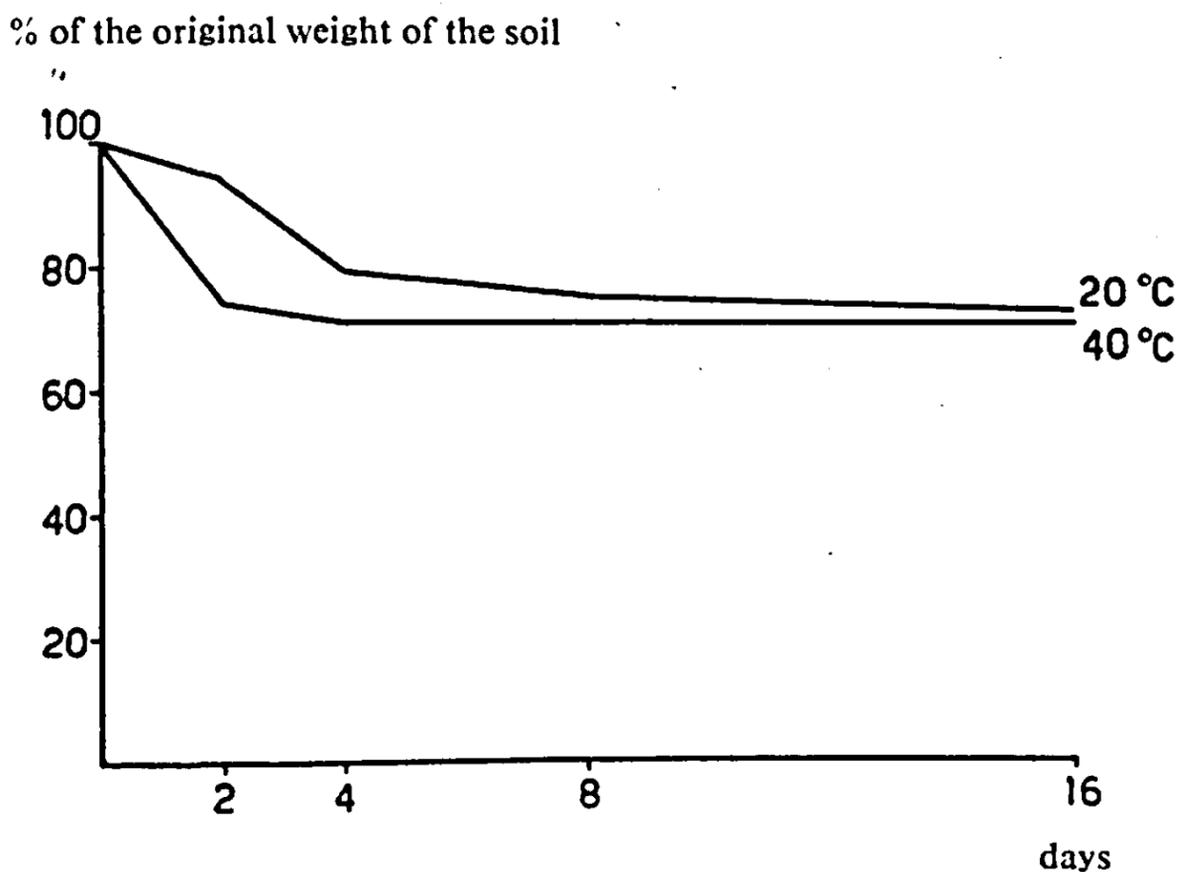


Graph 3

The occurrence of root-hair infection in infected soil, dried prior to planting, indicated by the average number of zoosporangia per unit of rootlength at various dates after the beginning of the experiment. 0 = no infection.

Aantasting van de wortelharen in vooraf gedroogde, besmette grond, uitgedrukt in het gemiddelde aantal zoösporangia per eenheid van wortellengte op verschillende dagen na het begin van de proef. 0 = geen aantasting.

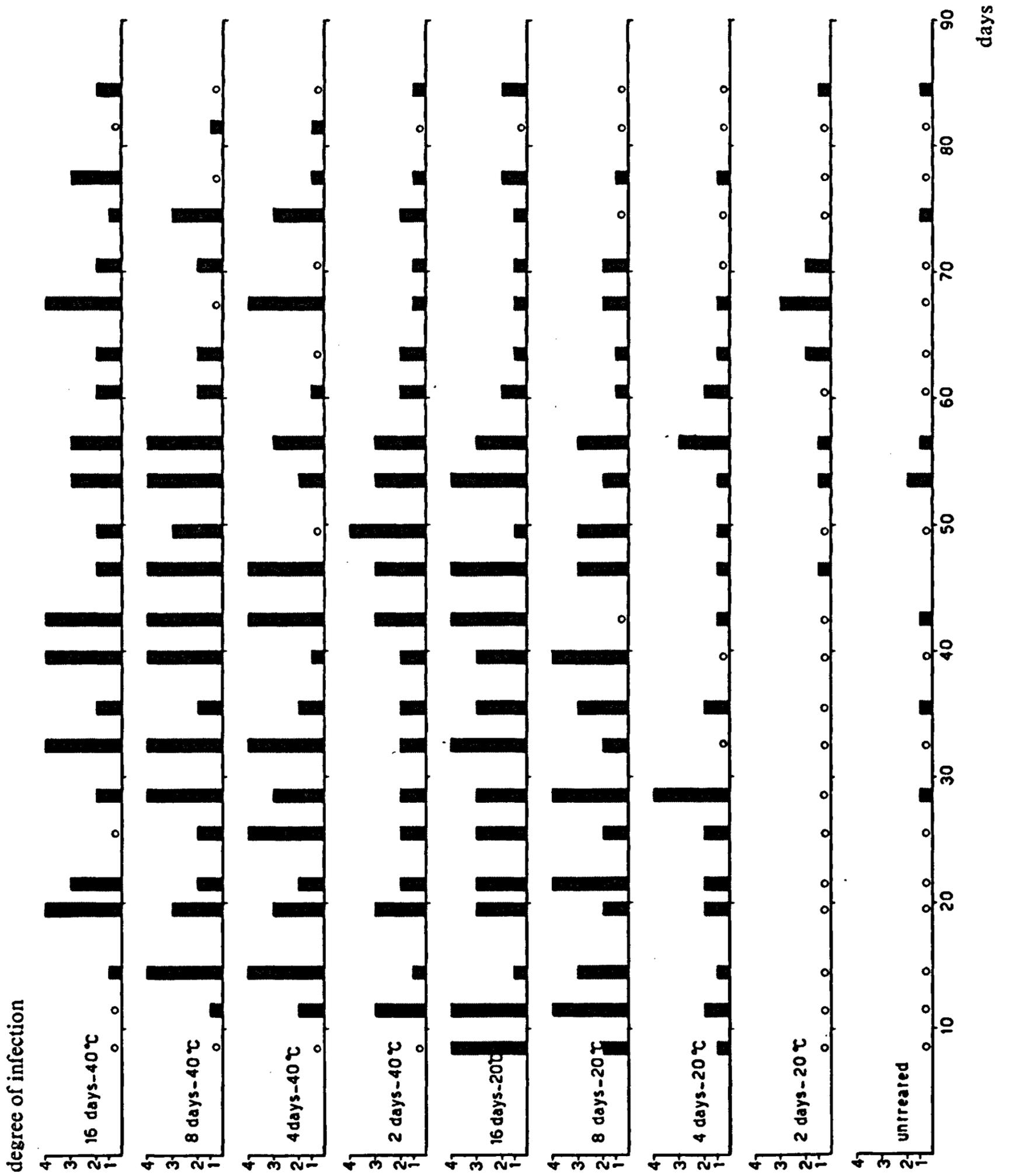
In an experiment to examine the degree of drying necessary to induce resting spore germination, heavily infected garden soil was kept moist at 15 °C for a long period. The soil was then divided into 8 equal volumes and dried at 2, 4, 8 and 16 days in electric hot-air ovens at temperatures of 20 and 40 °C. The loss in weight which occurred after drying is shown in graph 4 for the eight portions of soil used. At 20 °C the soil was air-dry after 8 days and at 40 °C after 4 days.



Graph 4

Decrease in weight of moist soil as a result of drying for 2, 4, 8 and 16 days at temperatures of 20 and 40 °C respectively. The weight is indicated in percentages of the original weight of the moist soil.

Gewichtsverlies van vochtige grond tengevolge van drogen bij een temperatuur van resp. 20 en 40 °C gedurende 2, 4, 8 en 16 dagen. Het gewicht is uitgedrukt in percenten van het oorspronkelijke gewicht van de vochtige grond.



Graph 5

The influence of the intensity of previous drying of infected, moist soil on the occurrence of root-hair infection.

0 no infection
 1 sporadic "
 2 slight "
 3 moderate "
 4 heavy "

De invloed van de intensiteit van een voorafgaande droging van vochtige, besmette grond op de aantasting van de wortelharen.

0 geen aantasting
 1 sporadische "
 2 geringe "
 3 matige "
 4 sterke "

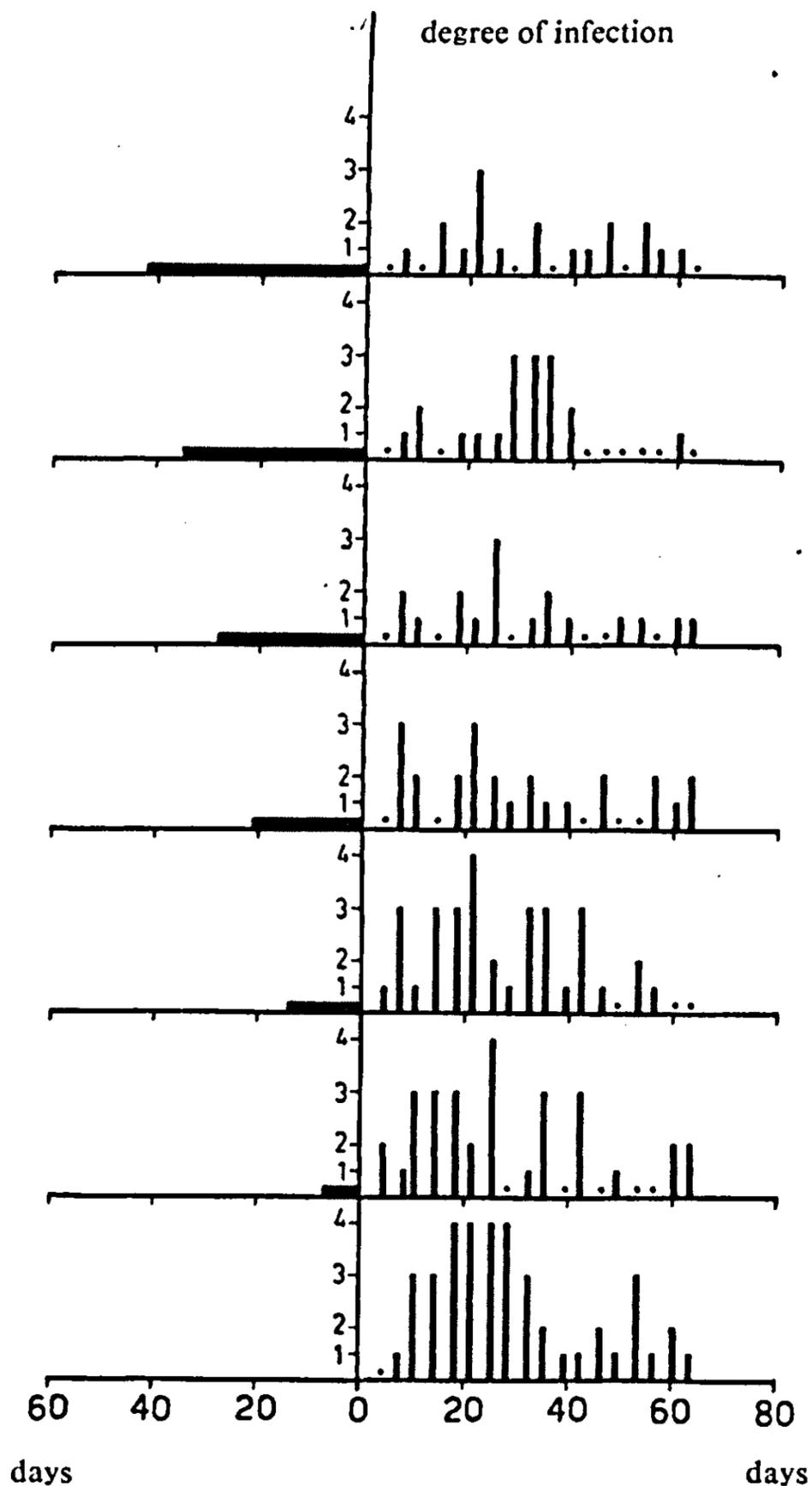
Root-hair infection of tomato seedlings was studied in the pre-dried soils and heavily infected moist soil, stored continuously at 15 °C, served as a control. The degree of infection was assessed as either heavy, moderate, slight and sporadic and was based on the microscopical examination of six 1 cm. lengths of root per plant. Successive determinations of root-hair infection were made at intervals of 2–3 days. The results are shown in graph 5 where heavy, moderate, slight and sporadic infection levels are given as 4, 3, 2, and 1 respectively. Only slight infection was obtained in the control soil and in the soil dried at 20 °C for two days. Soil dried for four days at 20 °C produced a slight infection over the whole experimental period, while greater infection was obtained in soils dried at 20 °C for 8 and 16 days. Soil dried at 40 °C for 2 days produced more infection than in control soil and the amount of infection was further increased in soils dried for 4 days at 40 °C. At 40 °C however, no further increase in infection was obtained in soils dried for 8 and 16 days. Infection in soils dried at 20 °C commenced 9 days after the beginning of the experiment but as is shown in graph 5, in soils dried at 40 °C infection commenced from 3–7 days later than in soils dried at 20 °C. The end of the infection period is not sharply marked decreasing in soils dried at 20 °C after 60 days and decreasing in soils dried at 40 °C somewhat sporadically. A summary of the results of the experiment is given in table 3.

TABLE 3. Frequency of root-hair infection in relation to soil treatment.
Frequentie van aantasting der wortelharen in verband met de behandeling van de grond.

Number of times the infection was diagnosed as <i>Aantal malen, dat de aantasting werd beoordeeld als</i>	absent <i>ontbrekend</i>	sporadic <i>sporadisch</i>	slight <i>gering</i>	moderate <i>matig</i>	heavy <i>sterk</i>
untreated <i>onbehandeld</i>	15	6	1	0	0
2 days at 20°C	16	4	2	1	0
4 " " "	6	9	6	1	1
8 " " "	4	3	7	5	4
16 " " "	1	6	3	7	6
2 " " 40°C	2	5	9	6	1
4 " " "	5	4	4	4	6
8 " " "	4	2	6	2	8
16 " " "	4	2	8	3	5

The experiment shows that resting spores do not germinate readily in soil that has been moist for a long period. Evidence is also provided that the spore germination may be considerably encouraged by drying the soil e.g. at 20 °C for 4 and 8 days and at 40 °C for 2 days. It will be seen from graph 4 that these periods of drying correspond with the time required to produce air-dry soil conditions. Indications were also obtained that the spore germination is influenced by the intensity of drying for rapid drying at a temperature of 40 °C appeared to delay the commencement of infection when compared with infection in soils dried more slowly at 20 °C.

In a further experiment to test the effect on spore germination of soil moisture relations, tomato seedlings were planted in moistened, previously dried soil as in the previous experiment and also in soils that had been dried, moistened and stored in moist conditions for 1–7 weeks prior to planting. The degree of root-hair infection was determined as in the previous experiment and the results are shown in graph 6. The greatest infection occurred in the soil which had not



Graph 6

The occurrence of root-hair infection in infected soil, dried and then kept moist for a varying number of days before planting. The horizontal lines on the abscissa on the left of the ordinate indicate the number of days the soil had been kept moist.

- no infection
- 1 sporadic „
- 2 slight „
- 3 moderate „
- 4 heavy „

Aantasting van de wortelharen in vooraf gedroogde grond, die gedurende verschillende aantallen dagen voor het inplanten werd vochtig gehouden. De horizontale lijnen op de abscis links van de ordinaat geven het aantal dagen aan, dat de grond vochtig werd gehouden.

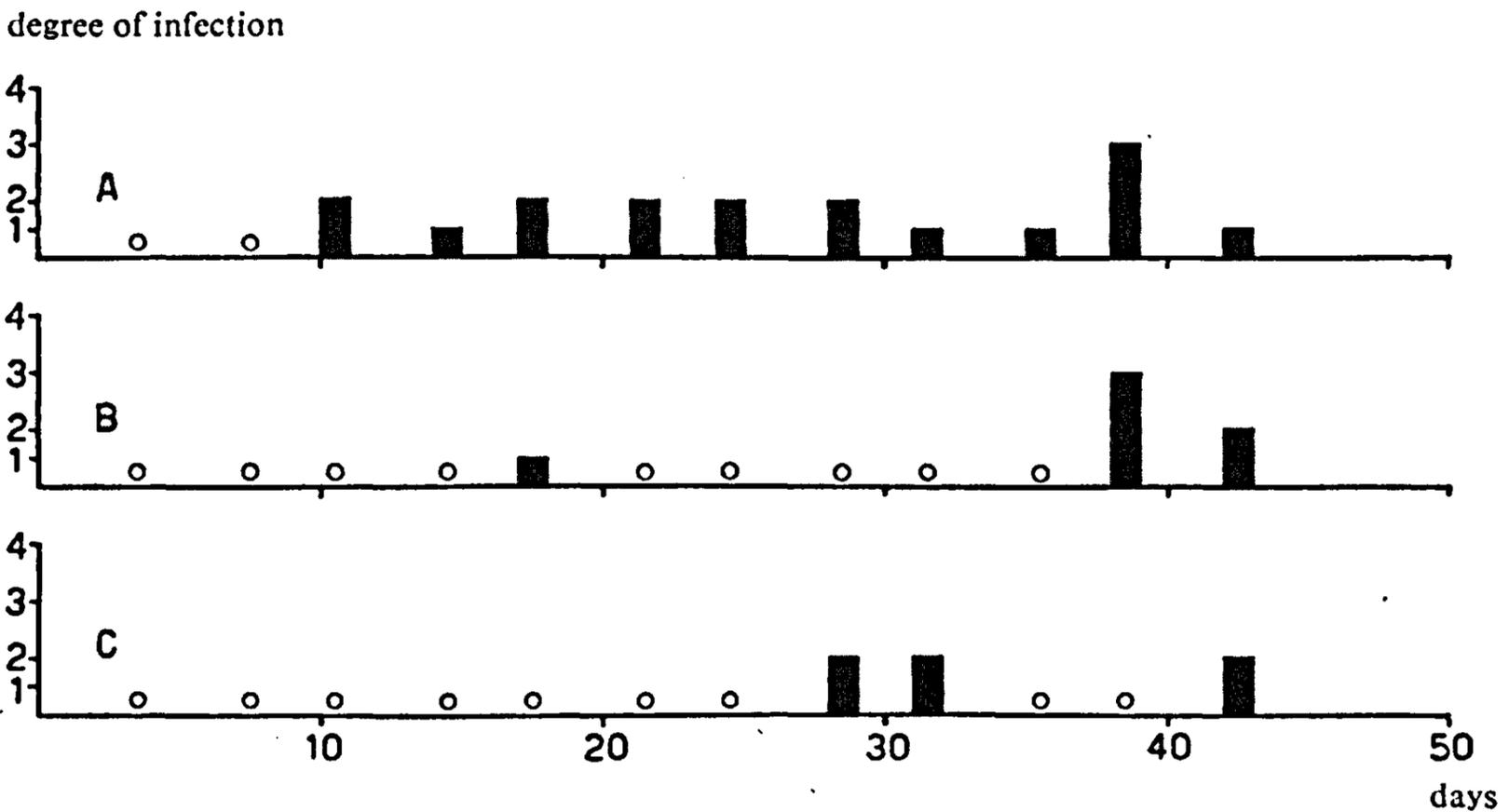
- geen aantasting
- 1 sporadische „
- 2 geringe „
- 3 matige „
- 4 sterke „

been stored in a moistened condition after drying. In soils stored in moistened conditions after drying however, successively less infection was obtained with increasing lengths of storage.

The experiment shows that the infectivity of previously dried soil containing

resting spores may be reduced under conditions of moist storage and especially when the soil is moistened for a period of more than two weeks.

The influence of freezing on the germination of the resting spores was investigated in infected soil that was kept moist for several months. Heavily infected moist garden soil was stored at -24°C and at air-dry laboratory temperatures for 5 days respectively. Untreated infected soil kept moist served as a control. Tomato seedlings were grown in the three soils and assessment of root-hair infection was made as in other experiments. The results of the experiment are shown in graph 7. Greater root-hair infection was obtained in the previously dried soil than in the control and frozen soils. It was also concluded that freezing did not encourage the germination of resting spores.



Graph 7

The influence of various treatments of infected soil on the occurrence of root-hair infection.

A. air-dried.

B. kept moist for a long period.

C. kept moist for a long period and then kept at -24°C for 5 days.

0 no infection

1 sporadic „

2 slight „

3 moderate „

4 heavy „

De invloed van verschillende behandelingen van besmette grond op de aantasting van de wortelharen.

A. aan de lucht gedroogd.

B. gedurende lange tijd vochtig gehouden.

C. gedurende lange tijd vochtig gehouden en daarna gedurende 5 dagen bij -24°C bewaard.

0 geen aantasting

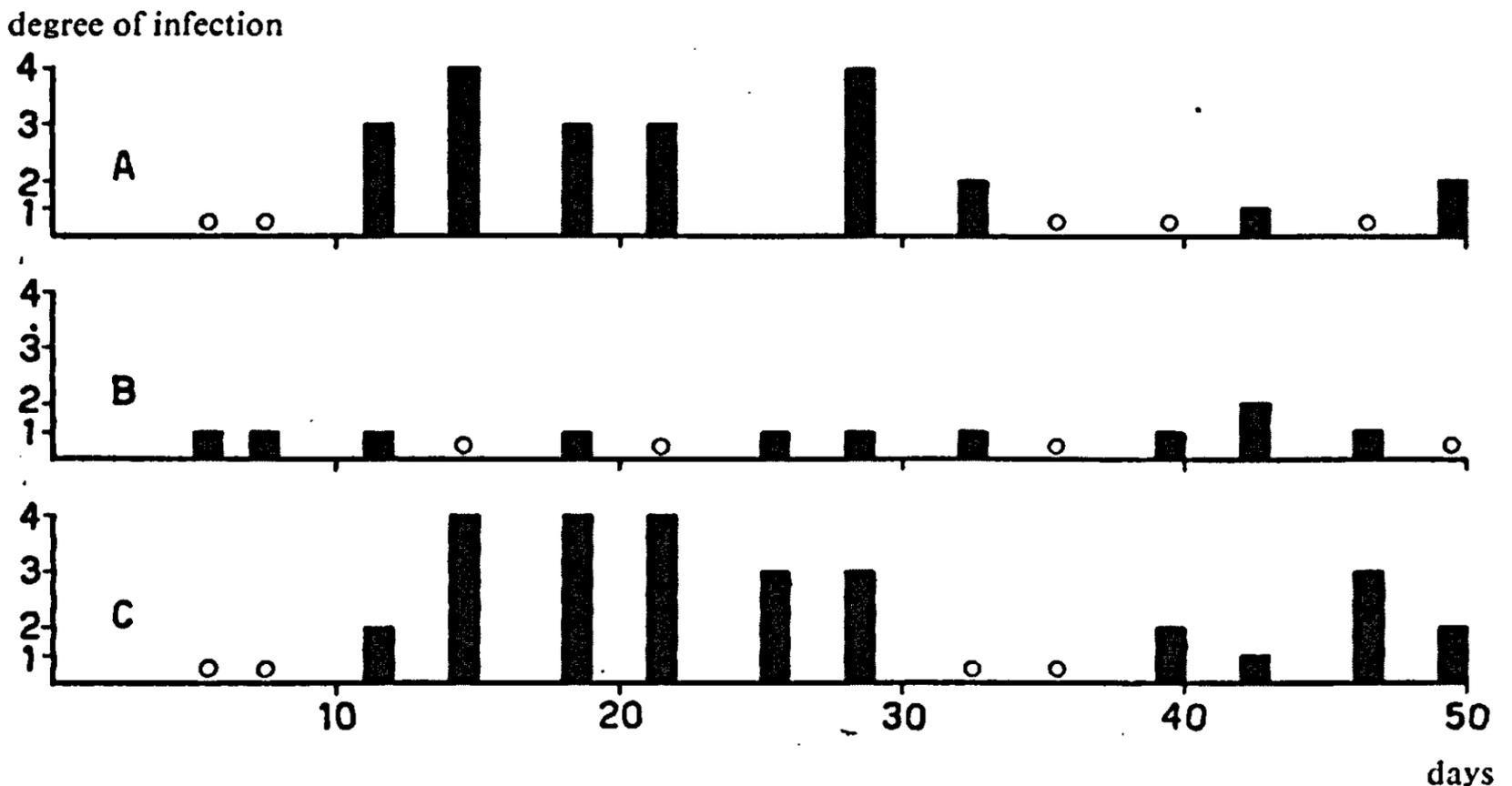
1 sporadische „

2 geringe „

3 matige „

4 sterke „

The viability of the resting spores was investigated by storing dry infected soil at -24°C and at 18°C for 56 days and moist infected soil at 18°C . The effect of the three treatments on resting spore germination was measured by the root-hair infection technique and the results of the experiment are given in graph 8. The germination of the resting spores was reduced when stored in moist soil at 18°C for 56 days while germination of resting spores stored in frozen soil differed little from that of the resting spores stored in air-dry soil at 18°C .



Graph 8

The influence of various treatments of infected soil on the occurrence of root-hair infection.

A. air-dried.

B. kept moist for 56 days.

C. air-dried and kept at -24°C for 56 days.

0 no infection

1 sporadic "

2 slight "

3 moderate "

4 heavy "

De invloed van verschillende behandelingen van besmette grond op de aantasting van de wortelharen.

A. aan de lucht gedroogd.

B. gedurende 56 dagen vochtig gehouden.

C. aan de lucht gedroogd en gedurende 56 dagen bij -24°C . bewaard.

0 geen aantasting

1 sporadische "

2 geringe "

3 matige "

4 sterke "

D. Discussion

In the life-cycle of *S. subterranea*, the zoospores developed from the zoosporangia in roots, initiate tuber infection so that the conditions favouring zoospore formation may also favour tuber infection.

In the above study it was shown that the conditions favouring root-hair infection in soil containing resting spores was a period of drought, during which the soil became air-dry, followed by wetting of the soil.

Soils containing resting spores were shown to lose their infectivity to tomato seedling roots when continuously stored in a moist condition in the absence of a host plant. It is not known whether, under these conditions, the spores gradually lose their ability to germinate or whether, by successive germination, the population of ungerminated spores becomes progressively smaller. However, from the results of the experiments on the influence of the quantity of nutrient solution on root-hair infection – indicating that the germination of resting spores is stimulated by the presence of the roots of a host plant – it may be concluded that the first assumption is correct.

CHAPTER 3

THE INFLUENCE OF SOIL TEMPERATURE AND HYDROGEN-ION CONCENTRATION ON ROOT-HAIR INFECTION

A. Introduction

KARLING (1942) showed that Powdery Scab is favoured by fairly low temperatures and that the soil pH apparently had no influence on tuber infection. In the present study the relationship of root-hair infection to soil temperature and pH have been studied.

B. Materials and Methods

The influence of temperature on root-hair infection was investigated in Wisconsin tanks. Shallow pans (10 cm. high) were filled with white sand and hung in the tanks. Three 8 cm. flower-pots, were filled with previously dried, infected garden soil, and sunk in each pan up to the rim. The infected soil in the pots was thoroughly moistened and tomato seedlings were planted in each pot. The sand surrounding the pots was kept moist to ensure even heat conduction. Periodically plants were removed for the examination of root-hair infection, in the manner previously described.

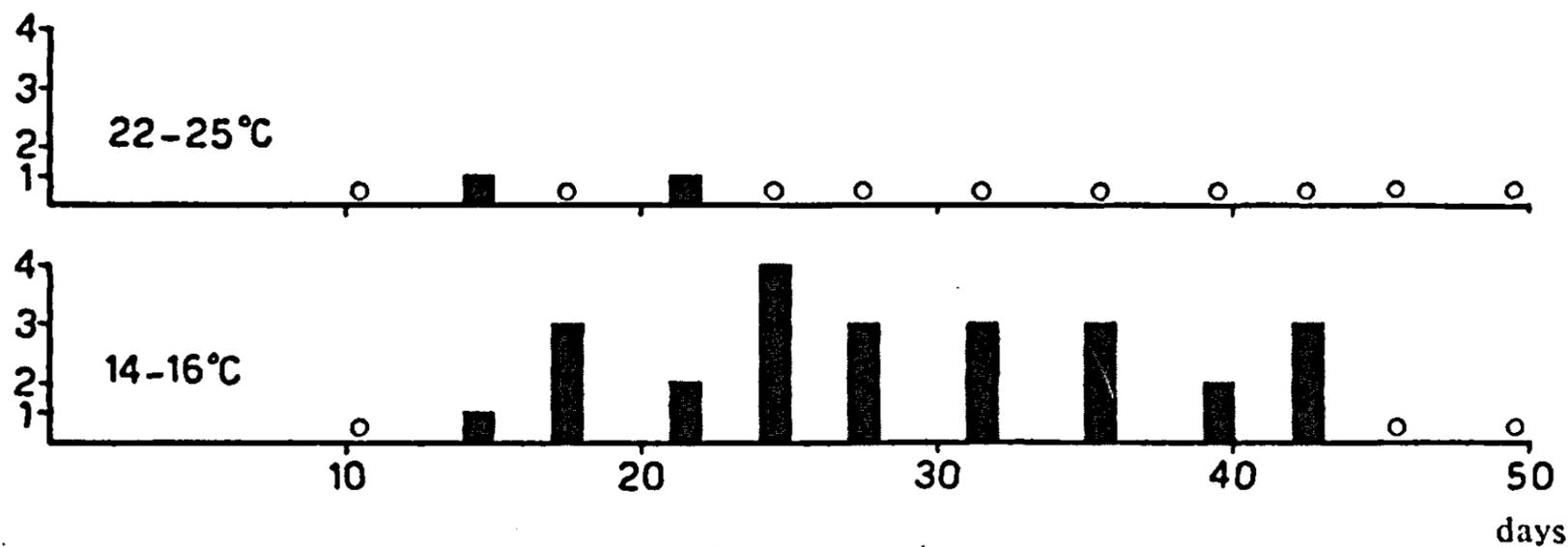
The effect of soil pH on root-hair infection was studied using heavily infected garden soil which was divided into four portions and adjusted to various pH values. To produce the desired pH values, the buffering capacity of the soil was determined. 10 gm. portions of the infected soil were mixed with 10, 5 and 2.5 ml. of 0.1 N sulphuric acid and 10, 5 and 2.5 ml. of a saturated solution of calcium hydroxide. Before mixing, the 5 and 2.5 ml. solutions were made up to 10 ml. with water. The treated soils were placed in tumblers and after 24 hours their pH value was determined. A titration curve was made from which the amounts of acid and base necessary to add to the soil to produce a desired pH value could be determined. The infected soil was then divided into four portions and three portions were adjusted to pH values of about 5, 6 and 8. The pH of one unadjusted portion was 6.8. Four 8 cm. flower pots were filled with the four different portions and placed in a glass-house at 18–20 °C. Tomato seedlings were planted in each pot and root-hair infection was determined as in previous experiments. The pH of the soils was determined at the beginning of the experiment and then successively at weekly intervals.

C. The influence of temperature on root-hair infection

The experiments were carried out in two Wisconsin tanks which maintained soil temperatures of 14–16 °C and 22–25 °C respectively. The degree of root-hair infection obtained is shown in graph 9.

At a soil temperature of 14–16 °C the root hairs became moderately infected 15–43 days after the beginning of the experiment. Except for the 15th and the

degree of infection



Graph 9

The influence of soil temperature on the occurrence of root-hair infection.

0 no infection
 1 sporadic „
 2 slight „
 3 moderate „
 4 heavy „

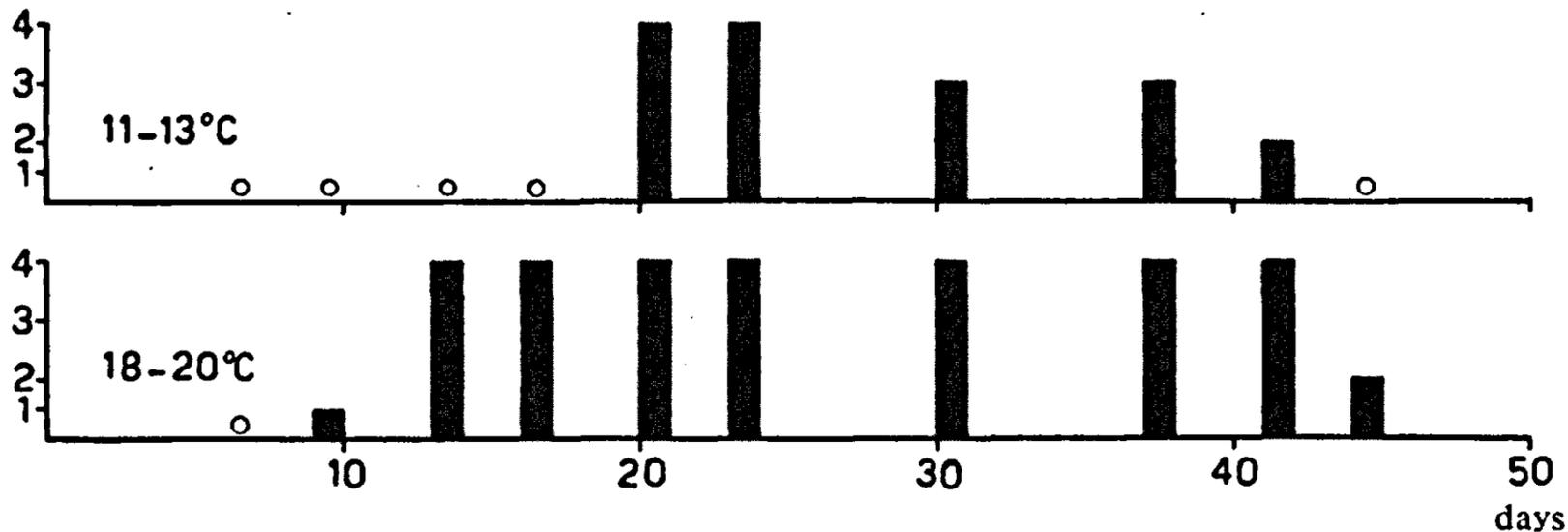
De invloed van de grondtemperatuur op de aantasting van de wortelharen.

0 geen aantasting
 1 sporadische „
 2 geringe „
 3 matige „
 4 sterke „

22nd day when zoosporangia were occasionally found, the root hairs remained healthy at soil temperatures between 22–25 °C. The experiment was repeated with soil temperatures of 18–20 °C and 11–13 °C respectively. The results of the experiment are shown in graph 10. At a soil temperature of 18–20 °C the root hairs became severely infected 10–45 days after the beginning of the experiment, while at 11–13 °C infection occurred between 20 and 43 days and was less severe than in the soil at 18–20 °C.

The maximum, optimum and minimum temperatures for root-hair infection therefore are 22–25 °C, 14–20 °C and below 11 °C respectively.

degree of infection



Graph 10

The influence of soil temperature on the occurrence of root-hair infection.

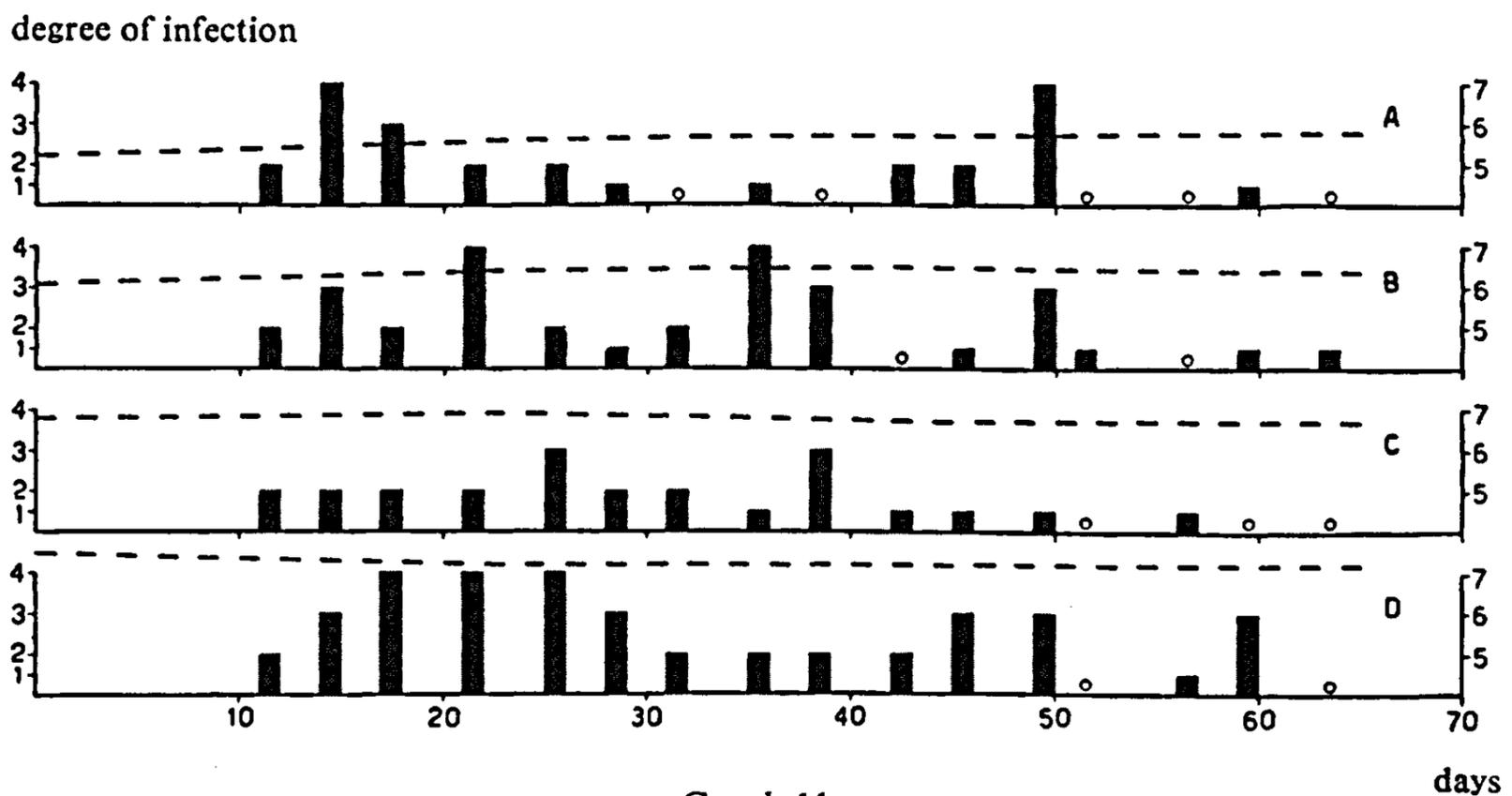
0 no infection
 1 sporadic „
 2 slight „
 3 moderate „
 4 heavy „

De invloed van de grondtemperatuur op de aantasting van de wortelharen.

0 geen aantasting
 1 sporadische „
 2 geringe „
 3 matige „
 4 sterke „

D. The influence of pH on root-hair infection

The results of the experiments on the influence of soil pH on root-hair infection are shown in graph 11. The change in pH value of the soils is also indicated on the graph. During the 64 days of the experiment the pH value of the soils changed only slightly. Root-hair infection occurred between pH values 5.2–7.5. The rate of root-hair infection in the untreated soil (pH 6.7–6.9) was slightly less than in most of the previous experiments in which previously dried soil was used. In the soils with pH values of 5.2–5.8 and 6.1–6.4 infection was relatively slight. Although no statistical treatment of the differences in disease intensity in the various soils has been possible, it appeared that root-hair infection was heaviest between pH 7.2 and 7.5.



Graph 11

The influence of the hydrogen-ion concentration of the soil on the occurrence of root-hair infection. The dotted line indicates the course of the pH value.

A = pH 5.2–5.8

B = pH 6.1–6.4

C = pH 6.7–6.9

D = pH 7.2–7.5

0 no infection

1 sporadic „

2 slight „

3 moderate „

4 heavy „

De invloed van de zuurgraad van de grond op de aantasting van de wortelharen. De stippellijn geeft het verloop van de pH aan.

0 geen aantasting

1 sporadische „

2 geringe „

3 matige „

4 sterke „

E. Discussion

Little is known of the influence of the temperature on root-hair infection in the *Plasmodiophorales*. AYERS (1944) tested the influence of soil temperature on root-hair infection of *P. brassicae* and found that the minimum, optimum and maximum temperatures for infection were 12–14 °C, about 21 °C and more than 33 °C.

Comparison with the results of the present study indicates that though the optimum infection temperatures of *P. brassicae* and *S. subterranea* differ only

slightly, *S. subterranea* has lower maximum and minimum temperatures for infection than *P. brassicae*. AYERS found that the incubation period for root-hair infection with *P. brassicae* decreased with increase in temperature and that the shortest incubation period was 2–2½ days. The shortest incubation period for root-hair infection in the soil by *S. subterranea* was found to be 10 days. The incubation period of *S. subterranea* was also found to decrease with an increase in temperature from 11–20 °C (Table 4). The incubation period at soil temperatures of 18–20 °C was shorter than that at 22–25 °C but is not considered to be significant since at the latter temperature the root-hair infection was near the maximum temperature for infection and was of sporadic occurrence only.

TABLE 4. The influence of soil temperature on the incubation period of root-hair infection.
De invloed van de grondtemperatuur op de incubatietijd van de aantasting der wortelharen.

Temp. °C	Incubation period in days <i>Incubatietijd in dagen</i>
11–13	21
14–16	15
18–20	10
22–25	15

The influence of soil pH on root-hair infection was investigated by SAMUEL & GARRETT (1945) with *P. brassicae*. They found that root-hair infection may be practically inhibited in an alkaline soil, thus supporting the distribution of Club Root as found in the field. In *S. subterranea* however, root-hair infection is not inhibited by an alkaline soil and the incidence of the infection seems to be largely independent of soil pH. These results are in agreement with the field experience that the occurrence of Powdery Scab appears to be independent of soil pH.

CHAPTER 4

THE INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON POTATO ROOT AND TUBER INFECTION

A. Introduction

OSBORN (1911) recorded the difficulty of transmitting Powdery Scab from both diseased tubers and contaminated soil to healthy potatoes grown under different soil conditions of moisture and temperature. KARLING (1942) described the conditions that favoured Powdery Scab in the field as heavy rainfall, low temperatures, damp, poorly drained and water-logged soils. Similar experience is found in the Netherlands where the disease mainly occurs in cool, wet summers.

Under experimental conditions RAMSEY (1918) obtained tuber infection in moist soil at 14 °C. In the present study conditions similar to those used by RAMSEY were employed. Potato plants were grown in moist, heavily infected soil in an air-conditioned glass-house at 18 °C, but only slight infection was obtained. Although experiments with tomato plants had shown that previous drying of infected soil increased spore germination, no tuber infection was produced by growing potato plants in heavily infected, previously dried soil.

Unexpected heavy tuber infection however, which occurred in potted plants grown in the open and in a glass-house where no special care was given to watering, prompted a study of the soil humidity relations to tuber infection.

B. The influence of soil humidity on tuber infection

Potato plants, variety Bintje, were raised from sprouted seed potatoes in pots containing sterilized garden soil. When 6 weeks old the plants were removed from the pots and after adhering soil had been removed the plants were replanted in 25 cm. pots containing dried, infected garden soil. After replanting, six pots were waterlogged, drained and plunged into a second larger pot containing moist peat-dust. In this way the soil was prevented from drying quickly. Three pots received no further watering while three pots were kept moist throughout the experiment by watering regularly. The pots were placed in a glass-house at 16–20 °C. Three weeks after the beginning of the experiment the tubers and roots of one plant of both series were removed and examined. The roots of the plant grown in soil that was kept constantly moist were slightly infected while the tubers had remained healthy. The roots of the plant grown in soil that was not watered after the beginning of the experiment were severely infected and occasional tuber infection had occurred. Five weeks after the beginning of the experiment one plant of each series was again examined. The roots of the watered plant were found to be moderately infected and of the four tubers formed two tubers had developed one small wart and two tubers showed several early stages of infection. The roots of the unwatered plant were severely infected and had developed numerous large warts and of four tubers formed two tubers had developed numerous and two tubers had developed occasional warts respectively. Six weeks after the beginning of the experiment, the remain-

ing two plants of each series were examined. The roots of the watered plant were moderately infected and had developed small warts while ten tubers produced by the plant were healthy. The roots of the unwatered plant were severely infected and especially the roots formed in the upper parts of the pots which had developed large warts. One tuber was severely infected, two tubers were slightly infected and four tubers were healthy.

The results of the experiment show that root and tuber infection may be considerably controlled by the soil moisture content. In the presence of continuous high soil moisture Powdery Scab infection may be largely prevented. The results obtained in the unwatered series show that the period of incubation for root and tuber infection may be less than three weeks.

The above experiment was repeated using two similar quantities of heavily infected soil kept respectively moist and dry for six weeks prior to planting. The soils were filled into six pots and planted with potato plants as previously described. After planting, the soils of both series were waterlogged but received no further water during the remainder of the experiment. Two weeks after the beginning of the experiment the soil had begun to dry out and one plant was examined from each series and found to be healthy. After four weeks the soils became air-dry and further plant development ceased and an examination of the remaining four plants was made. The roots of plants growing in the pre-dried and pre-moistened soils developed 32 and 61 and 6 and 13 warts respectively. No tuber infection occurred in the plants of either series, however.

An attempt was made to infect potato plants in infected soil without the difficulty of transplanting. The plants were raised in previously dried soil and grown with a minimum supply of water for two months. When sufficiently developed the plants were waterlogged and allowed to dry out as in previous experiments. In order to examine the influence of different soils clay as well as garden soil was used. A clay soil and a garden soil of pH 5 and 6.5 respectively, were mixed with equal quantities of heavily infected garden soil. The two soils mixtures were filled into 30 cm. pots and planted with potato plants. The pots were plunged into the soil of an unheated greenhouse and after two months they were waterlogged and transferred to a glass-house with a temperature of 18 °C. The soil temperature in the pots varied from 16–17 °C. Three weeks after waterlogging the soils dried and the plants began to die, when they were removed and examined. In the clay and garden soils the numbers of warts developed on the roots of six plants were 108, 120 and 101, and 52, 35 and 16 respectively. Of the number of tubers developed in the clay soil, 4 tubers were heavily infected, 2 tubers were moderately infected and 14 tubers remained healthy. In the garden soil 2 tubers were heavily infected, 4 tubers were moderately infected and 17 tubers remained healthy.

The results of the experiment confirmed the previous requirements found to be necessary for satisfactory infection and also showed that the amount of infection may vary in different types of soil.

C. Discussion

In attempts to infect potato tubers with Powdery Scab, RAMSEY grew potato plants in infected soil at 14, 17 and 24 °C. While he obtained some infection at 14 °C, no infection occurred at higher temperatures. In the present study severe infection was obtained at 16–17 °C under the conditions described. Since the

soil used by RAMSEY was kept moist from the previous season and was moistened at the beginning of his experiment his failure to obtain tuber infection at 17 °C may be attributed primarily to the absence of previous soil drying.

The results of the present study largely agree with the results of field observations made by MELHUS, ROSENBAUM & SCHULTZ (1916). They examined the roots of potato plants growing in infected field soil at weekly intervals from the time the plants first appeared. The first infection was found on August 5, when very small warts were detected on the roots. On August 11, the infection of the roots had increased and some of the tubers also showed early stages of infection. On August 19 no new warts could be found while those previously present had matured and had broken exposing a mass of spore balls. They could not explain why new infections did not continue to develop, since numerous young roots were present and the soil conditions were apparently comparable with those which had existed earlier. To determine the time required for infection and the subsequent development of conspicuous warts, 12 plants with extensive root systems were washed and transplanted from clean to infected field soil on August 5, when infection was first observed. After transplanting the plants were well watered and shaded for 3–4 days. On August 19 three plants were removed and their roots examined, but only one wart was found. When the remaining nine plants were removed, on September 3 however, 3–11 warts were found on the roots of 6 plants. A similar experiment was begun on September 7, when plants of approximately the same age as those used on August 5 were transplanted from healthy to diseased soil. The plants were dug on October 1 and the root systems and tubers carefully examined, but no infection was found.

In another experiment to determine whether the plant must be of a certain age before infection can occur, 200 seed pieces were planted on July 26 in infected soil in the field. On August 20, when the plants were about 7.5 cm. high and had extensive root systems, 100 plants were dug and examined, but no infection was found, although in an adjoining row planted on May 26 the roots were generally infected, infection having occurred between August 1 and 15. On September 24 the remaining 100 plants were dug and examined, but no infection was found. The authors considered the failure of infection to the fact that the plants had not reached a susceptible stage.

It is interesting to note that these experiments were carried out in a particularly wet summer and that the soil moisture conditions were apparently similar both before and after August 19. It is possible that the infection observed on August 5, first occurred between July 15–22 since it has been shown in the present study that the incubation period for root and tuber infection is from 2–3 weeks. If the infection period ended on August 5 and persistent wet conditions prevented further infection after August 5, it is possible to understand the slight infection obtained in plants planted into infected soil on August 5. Furthermore, with the plants raised from seed pieces it is probable that little root development occurred between the time they were planted (July 26) and August 5 when the period of infection from the soil had probably ceased.

BOYD (1951) tested several clones of *Solanum curtilobum* and *S. tuberosum* for susceptibility to Powdery Scab. He grew plants in infected moist soil but experienced considerable difficulty in establishing tuber infection. It is hoped that the present study may provide future research on Powdery Scab with some of the conditions which must be observed if satisfactory tuber infection is to be obtained.

SUMMARY

The present study of *Spongospora subterranea* (WALLR.) LAGERH. is divided into two parts:

- (I) the morphology, cytology and life history of the fungus and
- (II) the relationship of environmental factors to infection.

PART I

1. From the resting spores zoospores develop which penetrate the root hairs and root-epidermal cells and develop into multi-nucleate plasmodia. The plasmodial nuclei are of the wheel type and divide promitotically.
2. Zoosporangia are initiated by the development of the plasmodium into segments with one or more nuclei. The segments undergo an akaryote stage and zoospores are formed after at least one mitotic division of the nuclei.
3. The zoospores are biflagellate and heterocont. In the presence of free moisture the zoospores are discharged from the mature zoosporangia into the surrounding water where some may fuse in pairs or threes. A nuclear fusion of the zoospores was not observed.
4. In early stages of tuber infection, amoebae and plasmodia are present in the cells of the tissue beneath the periderm. While causing necrosis of the infected cells, the causal organism penetrates into deeper located layers of the cortex. The tissue becomes necrotic following infection only in the beginning; later on, the infected cells are not killed but often enlarge and sometimes develop into giant cells. The nuclei of these enlarged cells often show signs of deformation. The infected cells may be stimulated to continuous cell division resulting in the formation of wart-like excrescences. During these divisions the fungus may be passively distributed among the newly formed cells.
5. As long as the host cells continue to divide, the fungus remains in the vegetative stage with the nuclei dividing promitotically. At the conclusion of cell division of the host amoebae coalesce to form plasmodia. The plasmodia then undergo an akaryote stage. After the akaryote stage there are two and probably three or more consecutive mitotic divisions of the nuclei, after which the plasmodia develop into spore balls.
6. Early stages of root infection involve necrosis of the cortical cells beneath the epidermis but later the infection is similar to that of tuber infection.
7. The development of the warts on the tubers is finally checked by a wound periderm developed beneath the infected spot. When the potatoes are lifted in early stages of infection however, the pathogen may spread in the sub-peridermal tissue around the originally infected spot, causing a dry rot. This ability of the fungus to migrate seems to be limited to the stage in development of the infection, in which the infected tissue has not broken through the skin and in which the fungus is still in the vegetative stage. In early stages of infection both conditions are mostly fulfilled.

PART II

1. There are indications that the resting spores only germinate in presence of the roots of a host plant. Moreover it appears that a previous drying of the resting spores stimulates their germination.
2. Under continued moist soil conditions, the resting spores gradually lose their ability to germinate. The air-drying of moist, infected garden soil is sufficient for the resting spores to regain their germinative power. Freezing of the moist soil had no effect on resting spore germination.
3. The resting spores in air-dry soil were not killed when exposed for 56 days to a temperature of -24°C . The maximum, optimum and minimum temperatures for root-hair infection were between $22-25^{\circ}\text{C}$, $14-20^{\circ}\text{C}$ and below 11°C respectively.
4. Within the limits of the investigation (pH 5.2-7.5) the hydrogen-ion concentration of the soil has little influence on the occurrence of root-hair infection.
5. Tuber and root infection is favoured by moist soil conditions in the earliest stages of infection and then after by gradual drying of the soil.
6. The incubation period of tuber and root infection is less than 3 weeks at a temperature of $16-20^{\circ}\text{C}$.

SAMENVATTING

Algemene inleiding. De aanleiding tot het onderzoek waren moeilijkheden, die in Nederland tengevolge van een aantasting door *Spongospora subterranea* (WALLR.) LAGERH. bij de export van aardappelen werden ondervonden. Deze deden zich voornamelijk voor bij de export naar landen, die de eis stellen dat er geen enkele aangetaste knol in de partij mag voorkomen.

Poederschurft, het ziektebeeld dat door *S. subterranea* wordt veroorzaakt, wordt vooral aangetroffen bij aardappelen, die afkomstig zijn van de zand- en dalgronden in het Noord-Oostelijk deel van ons land. De ernst van de aantasting en de omvang van optreden lopen van jaar tot jaar sterk uiteen en hangen nauw samen met de weersomstandigheden. In droge zomers is het vaak moeilijk enige aantasting te vinden, terwijl in normale, maar vooral in regenrijke zomers poederschurft in ernstige mate kan voorkomen. In het overige deel van het land komt de ziekte verspreid voor, maar heeft er weinig te betekenen.

Het doel van het onderzoek was aanvankelijk de bestrijdingsmogelijkheden en het verloop van de aantasting gedurende de bewaring te bestuderen. In de loop van het onderzoek kwamen echter feiten naar voren, die het noodzakelijk maakten de doelstelling te wijzigen. Deze werd nu het verkrijgen van een betere kennis van de ziekteverwekker en van de omstandigheden, die het optreden van poederschurft beheersen. In het eerste deel van de publicatie worden de morfologie, cytologie en levenswijze van *S. subterranea* behandeld; in het tweede deel is de invloed van enkele factoren op het tot stand komen van de aantasting nagegaan.

De kringloop van de ziekteverwekker. De schimmel overwintert in de vorm van sporenballen, zowel in de grond als in de poederschurftplekjes op aardappelknollen. Deze sporenballen zijn sponsvormige aggregaten van dikwandige rustsporen. De rustsporen kiemen met zoösporen. Deze infecteren de wortelharen en epidermiscellen van de wortel en ontwikkelen zich tot zoösporangia, die ook weer zoösporen vormen. Het is waarschijnlijk, dat deze zoösporen de knollen, wortels en stolonen aantasten, waardoor zich op de knollen de typische poederschurftplekjes en aan de wortels en stolonen wratten ontwikkelen. In het aangetaste weefsel verandert de schimmel in sporenballen, die bij vergaan van het weefsel vrijkomen.

Het zoösporangium en de zoösporen. De ontwikkeling van het zoösporangium is onderzocht bij worteltjes van aardappel- en tomatenplanten. Aantasting werd verkregen door watercultures van deze planten te besmetten met sporenballen. Uit binnengedrongen, éénkernige zoösporen ontwikkelen zich in jonge wortelharen en epidermiscellen van de wortel plasmodia ¹⁾. De kernen in de plasmodia delen zich op promitotische ²⁾ wijze. Het volgroeide plasmodium wordt door

¹⁾ Onder het plasmodium wordt verstaan het naakte, veelkernige zwamlichaam.

²⁾ Onder promitose wordt verstaan een vermoedelijk primitief type kerndeling, waarbij de kernwand en nucleolus intact blijven, de afzonderlijke chromosomen niet of moeilijk zijn te onderscheiden en de nucleolus zich door insnoering deelt.

wandjes in segmenten verdeeld en maakt een akaryotisch ¹⁾ stadium door, waarna de kernen zich minstens éénmaal op mitotische wijze delen. Tenslotte ontwikkelen de segmenten zich tot zoösporangia, waarvan het merendeel vier zoösporen bevat.

Zoösporen voor nader onderzoek werden verkregen door aangetaste wortels van tomatenplantjes uit zwaarbesmette grond af te spoelen en de zoösporen in een druppel water te laten vrijkomen. De zoösporen hebben één lange en één korte zweepdraad. In preparaten van zoösporen zijn echter ook exemplaren gevonden met het dubbele en in mindere mate ook met het drievoudige aantal zweepdraden. Waarnemingen met de phase-contrast microscoop hebben uitgewezen, dat deze samengestelde zoösporen het gevolg zijn van een versmelting van enkelvoudige zoösporen. Er zijn geen aanwijzingen verkregen, dat ook de kernen versmelten; in de onderzochte cytologische preparaten werden namelijk bij alle samengestelde zoösporen de kernen afzonderlijk aangetroffen. Aanvankelijk deed de versmelting van zoösporen denken aan het begin van een geslachtelijke fase, hetgeen echter niet kon worden bewezen door het ontbreken van waarnemingen over een kernversmelting. Om deze reden kon de versmelting van zoösporen slechts worden verklaard door aan te nemen, dat het hier een vegetatief proces betreft. In hoeverre de versmelting van essentieel belang is in de kringloop van de ziekteverwekker kon niet worden vastgesteld.

De aantasting van knollen, wortels en stolonen. Bij het onderzochte materiaal was de zwam voornamelijk door de lenticellen in de knollen binnengedrongen. In de aangetaste cellen werd de ziekteverwekker in de vorm van plasmodia en amoeben ²⁾ aangetroffen. Aanvankelijk breidt de aantasting zich in het weefsel onder de schil uit en veroorzaakt daarbij necrose van de aangetaste cellen. Vervolgens dringt de aantasting ook in dieper gelegen lagen van de schors door. In latere stadia gaat de aantasting niet meer met necrose van de aangetaste cellen gepaard; vanuit de necrotische cellen wordt het omliggende weefsel door amoeben geïnfecteerd. Dit gaat meestal samen met hypertrophie – waarbij soms „reuzencellen” ontstaan – maar de cellen blijven ondanks veelvuldig voorkomende misvormingen van de kernen in leven. Tenslotte worden de aangetaste cellen tot sterke celdeling geprikkeld, waardoor wratten worden gevormd. Bij deze celdelingen wordt de parasiet passief over de cellen van het nieuwgevormde weefsel verdeeld.

Bij de wortels gaat de aantasting samen met necrose van de cellen vlak onder de epidermis; overigens verloopt de aantasting bij de wortels op dezelfde wijze als bij de knollen.

Zolang bij de gastheercellen onder invloed van de aanwezigheid van de ziekteverwekker deling optreedt, blijft de zwam in het vegetatieve stadium, waarin de kernen zich promitotisch delen. Zodra bij de gastheercellen geen deling meer optreedt, versmelten de amoeben tot plasmodia, die daarna een akaryotisch stadium doormaken. Vervolgens delen de kernen zich, in ieder geval tweemaal, waarschijnlijk drie maal en misschien nog vaker, op mitotische wijze, waarna de plasmodia in sporenballen overgaan.

¹⁾ Het akaryotische stadium is een stadium in de ontwikkeling van het plasmodium, waarbij de kerninhoud zich weinig laat kleuren.

²⁾ De term „amoeben” wordt hier gebruikt voor lichaampjes, die meestal éénkernig zijn, zich waarschijnlijk door deling vermeerderen en die tenslotte tot plasmodia versmelten.

De ontwikkeling van de wratten op de knollen komt tot staan tengevolge van vorming van wondkurk onder de aangetaste plekjes. Worden de knollen echter in een jong stadium van aantasting geroid, dan verbreidt de ziekteverwekker zich meestal sterk in het weefsel vlak onder de schil rondom het aangetaste plekje, waardoor droogrot optreedt. Dit gebeurt vermoedelijk slechts in het stadium, waarin het aangetaste weefsel de schil nog niet heeft doorbroken en de schimmel nog in het vegetatieve stadium verkeert.

Het kiemen van de rustsporen. Uit infectieproeven met tomatenplantjes in watercultures met verschillende hoeveelheden voedingsvloeistof bleek, dat de aantasting van de wortelharen – waarbij dus zoösporangia worden gevormd – vroegtijdiger en in sterkere mate optreedt, naargelang de hoeveelheid voedingsvloeistof geringer is. Op grond hiervan wordt verondersteld, dat wortelsecreten een stimulerende invloed uitoefenen op het kiemen der rustsporen. Nadere proeven om de rustsporen met wortelsecreten van de aardappelplant tot kieming te brengen, hadden echter een negatief resultaat.

De invloed van verschillende behandelingen op het kiemen der rustsporen. Deze invloed werd onderzocht aan de hand van de mate van aantasting der wortelharen van tomatenplanten in besmette grond. Hierbij bleek, dat er minder rustsporen kiemen naargelang de grond langer in vochtige toestand verkeert. Het luchtdroog maken van de grond is voldoende om de rustsporen weer kiemkrachtig te maken. Er zijn aanwijzingen, dat het kiemen van de sporen wordt vertraagd, naarmate de grond intensiever wordt gedroogd.

Tevens werd nagegaan, wat er met de rustsporen gebeurt, indien de gedroogde grond na het bevochtigen niet direct, maar eerst na verloop van enige tijd met een waardplant wordt beplant. Hierbij bleek, dat er ook minder rustsporen kiemen, naarmate het langer duurt, voordat de grond na het bevochtigen wordt beplant; de sterkste aantasting werd verkregen, indien er niet meer dan twee weken verlieden tussen het bevochtigen van de grond en het beplanten. Vermoedelijk berust dit verschijnsel niet op het reeds kiemen van de rustsporen gedurende de afwezigheid van een waardplant, maar op het geleidelijk achteruitgaan in kiemkracht van de rustsporen na het bevochtigen.

Het bevriezen van langdurig vochtig gehouden besmette grond had geen invloed op de kiemkracht van de rustsporen.

In luchtdroge grond verdroegen de rustsporen een verblijf van 56 dagen bij een temperatuur van -24°C .

De invloed van de temperatuur en de zuurgraad van de grond op de aantasting der wortelharen. De invloed van de temperatuur is onderzocht aan de hand van de mate van aantasting der wortelharen van tomatenplanten in besmette grond, waarvan de temperatuur in Wisconsin-tanks op verschillende waarden werd gehouden. De maximum temperatuur voor het verkrijgen van een aantasting lag tussen 22° en 25°C en de optimum temperatuur tussen 14° en 20°C . De minimum temperatuur werd bij $11-14^{\circ}\text{C}$ nog niet bereikt.

De invloed van de zuurgraad is onderzocht door de mate van aantasting na te gaan in besmette grond, die met gebluste kalk of zwavelzuur op verschillende zuurgraden werd gebracht. Aantasting van de wortelharen werd zowel verkregen in een basisch als in een zuur milieu; binnen de grenzen van pH 7.5 en 5.2 had de zuurgraad weinig of geen invloed op de mate van aantasting.

De invloed van enkele factoren op het tot stand komen van de aantasting van knollen, wortels en stolonen. Binnen drie weken werd een ernstige aantasting van knollen en wortels verkregen door flink ontwikkelde aardappelplanten over te planten in vooraf gedroogde, besmette grond, die direct na het overplanten met water werd verzadigd. Nadien werd niet meer gegoten zodat de grond geleidelijk uitdroogde.

Dezelfde resultaten zijn verkregen door aardappelplanten met zeer weinig water op te kweken in vooraf gedroogde, besmette grond en deze zodra er voldoende knolontwikkeling was, te verzadigen met water en daarna geleidelijk te laten uitdrogen.

Bij een vergelijkende proef met kleigrond en tuinaarde werd bij dezelfde graad van besmetting van de grond en gelijke behandeling, in kleigrond een heviger aantasting verkregen dan in tuinaarde.

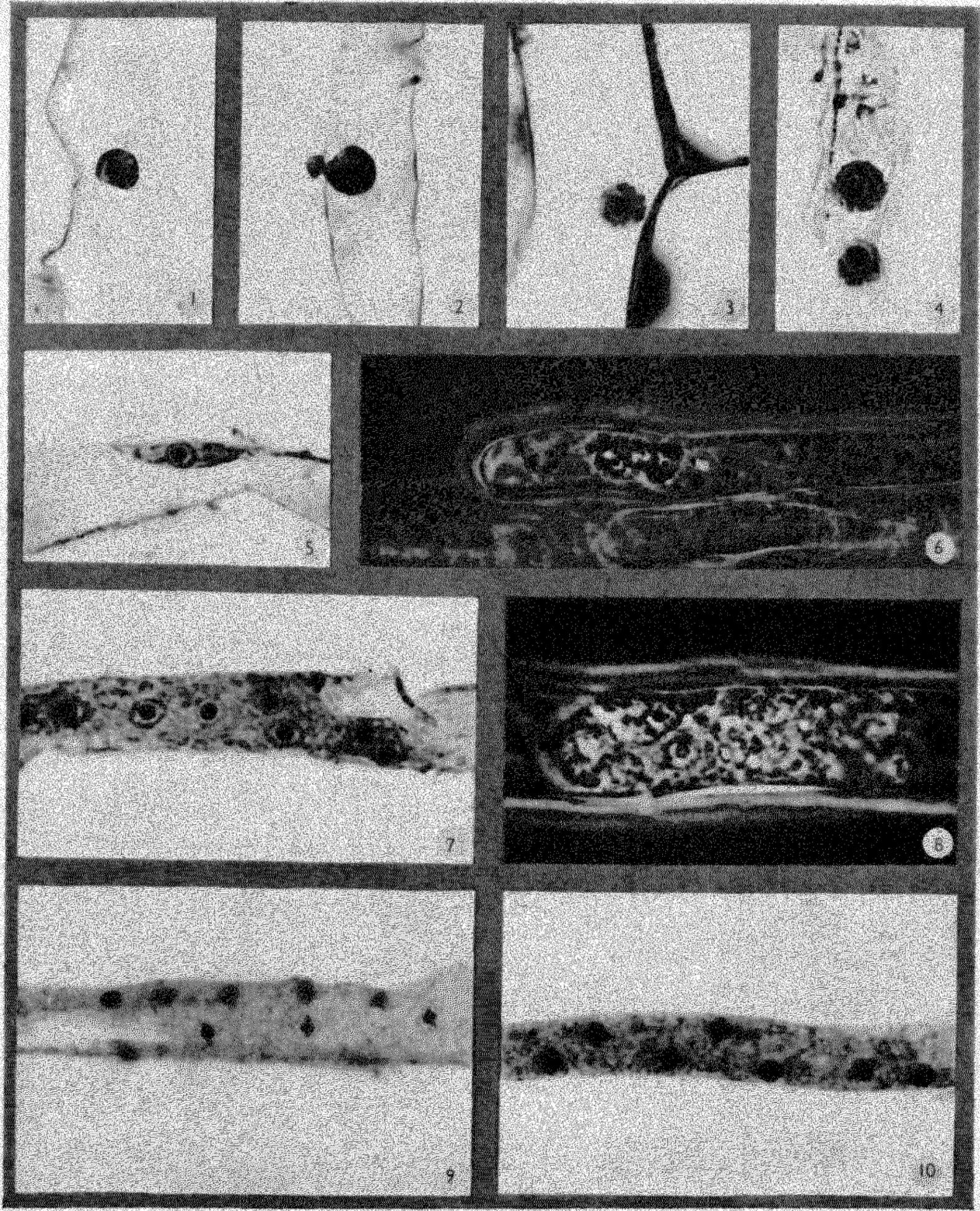
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PLATES

PLATE I

- Figs. 1, 2, 3, 4. Zoospores from resting spores as seen before penetration, during penetration of a root hair, after penetrating an epidermal cell of the root and after penetrating a root hair respectively. Stained with Heidenhain's haematoxylin. $\times 1172$.
Zoösporen afkomstig uit rustsporen, achtereenvolgens vóór het binnendringen, tijdens het binnendringen in een wortelhaar, na het binnendringen in een epidermiscel van de wortel en na het binnendringen in een wortelhaar.
- Fig. 5. A young plasmodium, which has developed from a zoospore, lying against the wall of a root hair. Stained with Heidenhain's haematoxylin. $\times 1172$.
Een jong plasmodium, dat zich heeft ontwikkeld uit een binnengedrongen zoöspore, liggende tegen de wand van een wortelhaar.
- Fig. 6. A young plasmodium in a root hair. Water mount. Phase contrast. $\times 540$.
Een jong plasmodium in een wortelhaar.
- Figs. 7, 8. Fully developed plasmodia as seen in a root hair. Stained with Heidenhain's haematoxylin and as observed in a water mount with the phase contrast microscope respectively. $\times 1172$.
Volledig ontwikkelde plasmodia in een wortelhaar.
- Figs. 9, 10. Metaphase of the promitotic divisions of the nuclei in the plasmodium. Stained with Heidenhain's haematoxylin. $\times 1172$.
Metaphase van de promitotische delingen van de kernen in het plasmodium.



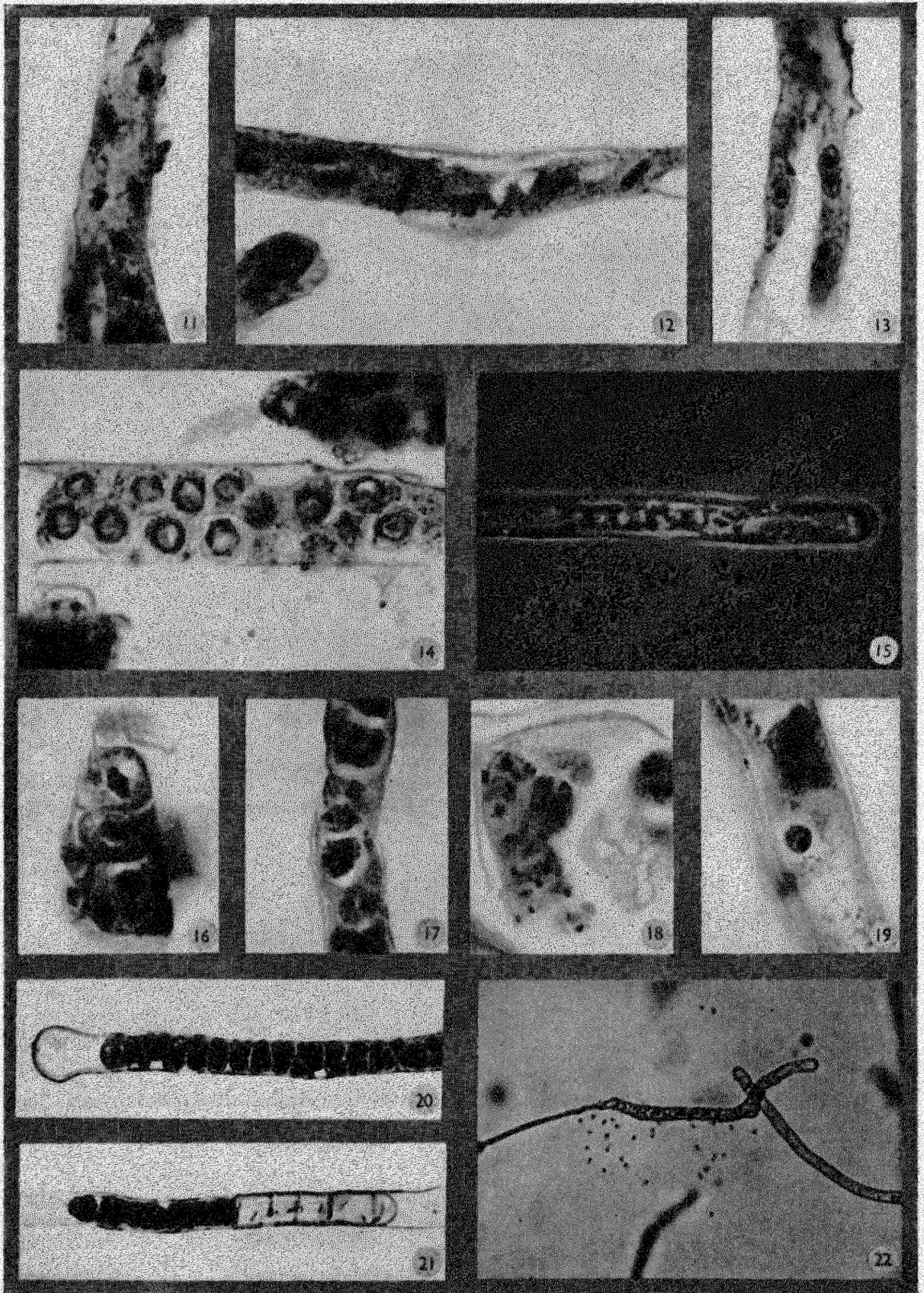
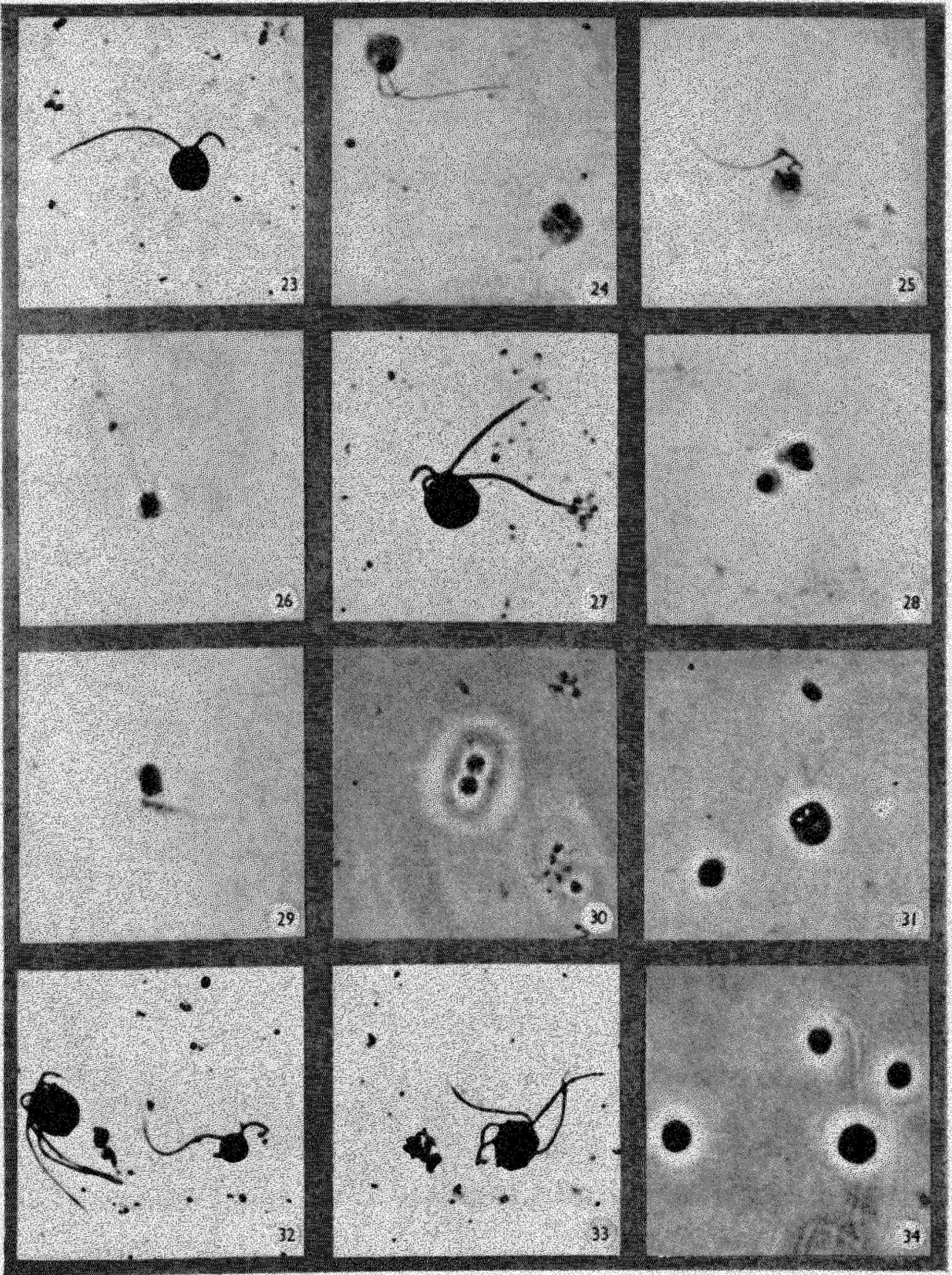


PLATE II

- Figs. 11, 12. Anaphase of the promitotic divisions of the nuclei in the plasmodium. Stained with Heidenhain's haematoxylin. $\times 1172$.
Anaphase van de promitotische delingen van de kernen in het plasmodium.
- Fig. 13. Telophase of the promitotic divisions of the nuclei in the plasmodium. Stained with Heidenhain's haematoxylin. $\times 1172$.
Telophase van de promitotische delingen van de kernen in het plasmodium.
- Fig. 14. A plasmodium in a root hair at the beginning of the segmentation. During the akaryote stage. Stained with Heidenhain's haematoxylin. $\times 1172$.
Een plasmodium in een wortelhaar bij het begin van de segmentatie tijdens het akaryotische stadium.
- Fig. 15. Akaryote stage of a plasmodium in a root hair. Water mount. Phase contrast. $\times 540$.
Akaryotisch stadium van een plasmodium in een wortelhaar.
- Figs. 16, 17. Metaphase and telophase stage of the nuclei in the young zoosporangia during mitosis. Stained with Heidenhain's haematoxylin. $\times 1172$.
Metaphase en telophase stadium van de mitotische delingen van de kernen in de jonge zoösporangia.
- Fig. 18. Several stages in the development of the nuclei of zoospores in the zoosporangia. Stained by Newton's iodine gentian-violet method. $\times 1172$.
Verschillende stadia in de ontwikkeling van de kernen van zoösporen in de zoösporangia.
- Fig. 19. A zoospore in a zoosporangium in a root hair. Stained with Heidenhain's haematoxylin. $\times 1172$.
Een zoöspore in een zoösporangium in een wortelhaar.
- Figs. 20, 21. A root hair containing mature zoosporangia and a root hair in which some zoosporangia are empty. Water mount. Stained slightly with neutral red. $\times 540$.
Een wortelhaar met rijpe zoösporangia en een wortelhaar met enkele, reeds lege zoösporangia.
- Fig. 22. Zoospore discharge from the zoosporangia occurring in a root hair. Water mount. $\times 125$.
Het vrijkomen van zoösporen uit zoösporangia in een wortelhaar.

PLATE III

- Figs. 23, 24. Zoospores from zoosporangia. Stained with Löffler's (Couch) method and with gentian violet (Cotner) respectively. $\times 1172$.
Zoösporen afkomstig uit zoösporangia.
- Figs. 25, 26. A zoospore with two blepharoplasts and a zoospore with two rhizoplasts respectively. Stained with gentian violet (Cotner). $\times 1172$.
Achtereenvolgens een zoöspore met twee blepharoplasten en een zoöspore met twee rhizoplasten.
- Fig. 27. A quadriflagellate zoospore. Löffler's (Couch) stain. $\times 1172$.
Een zoöspore met vier flagellen.
- Figs. 28, 29. A quadriflagellate zoospore with two separate nuclei. Stained with gentian violet (Cotner). $\times 1172$.
Het versmeltingsproduct van twee zoösporen, waarvan de kernen in fig. 28 afzonderlijk zichtbaar zijn en in fig. 29 elkaar gedeeltelijk bedekken.
- Fig. 30. A zoospore couple. Water mount. Phase contrast. $\times 756$.
Gepaarde zoösporen.
- Fig. 31. A quadriflagellate zoospore. Water mount. Phase contrast. $\times 756$.
Het versmeltingsproduct van twee zoösporen.
- Figs. 32, 33. A hexaflagellate zoospore. Stained with Löffler's (Couch) stain. $\times 1172$.
Een zoöspore met zes flagellen.
- Fig. 34. A hexaflagellate, a quadriflagellate and two biflagellate zoospores. Water mount. Phase contrast. $\times 756$.
Twee enkele zoösporen en het versmeltingsproduct van twee en van drie zoösporen.



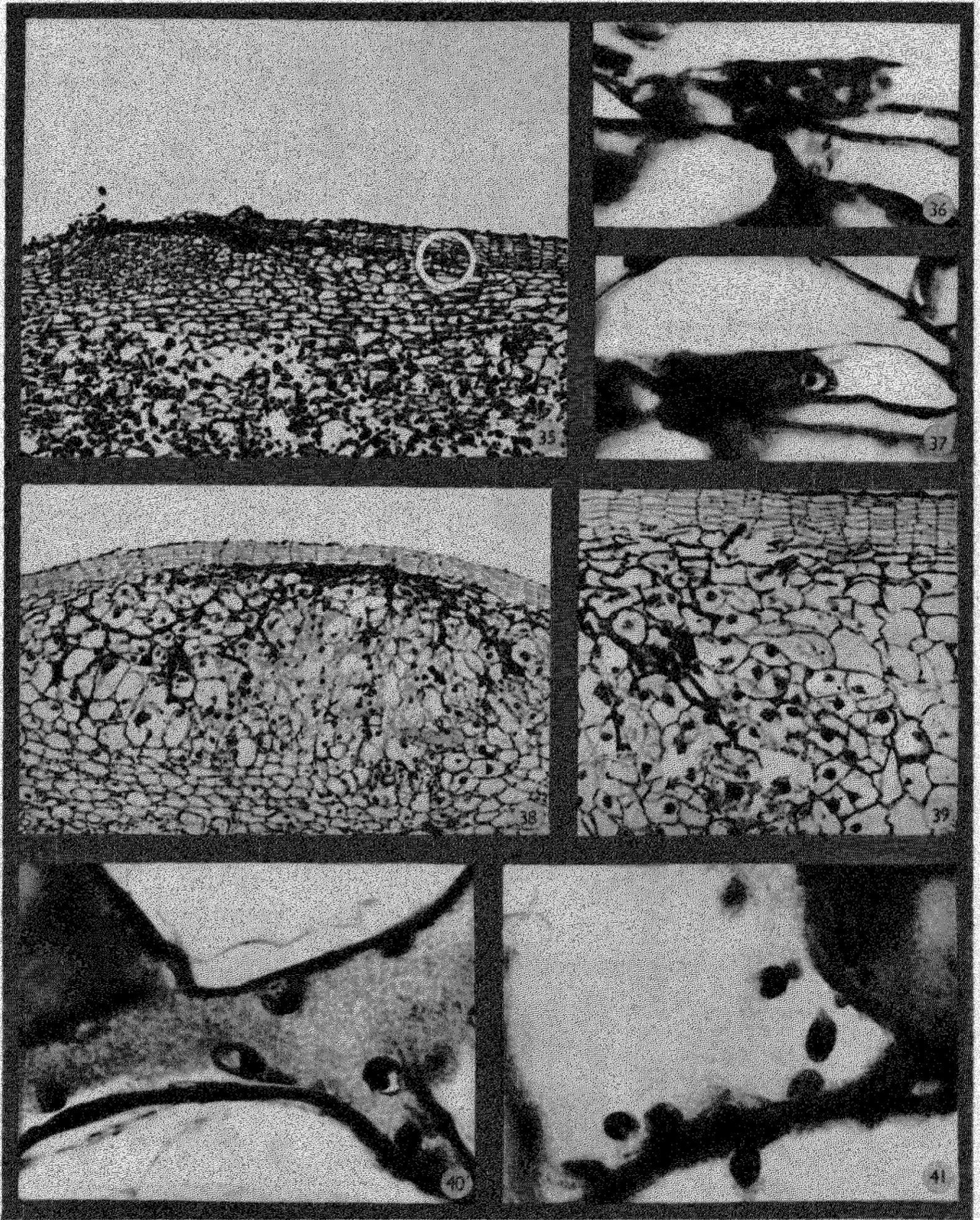
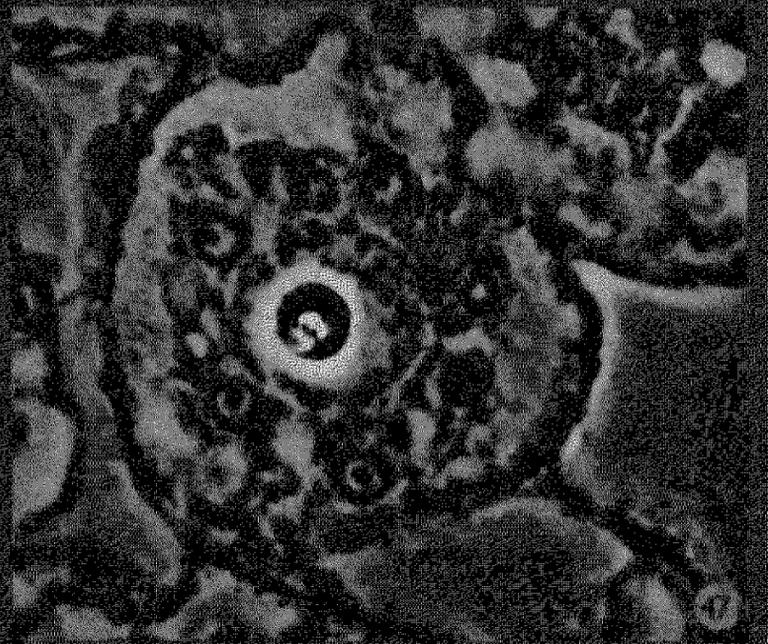
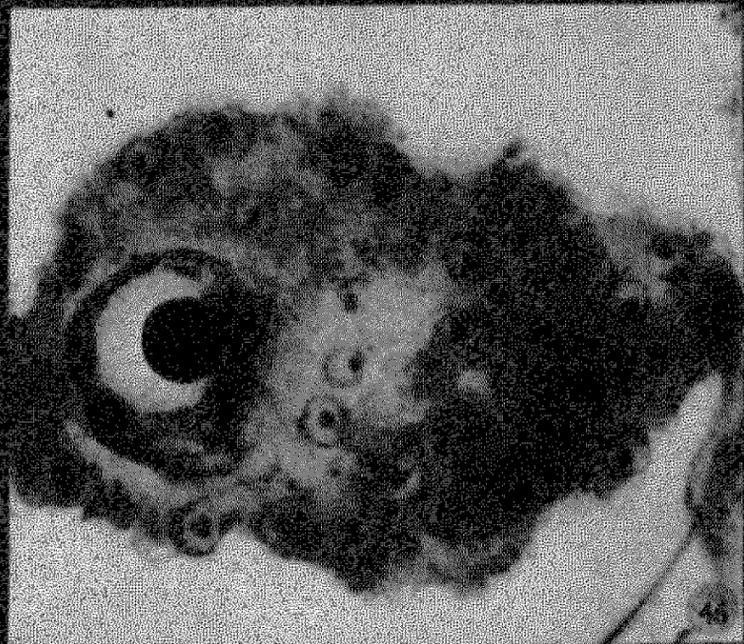
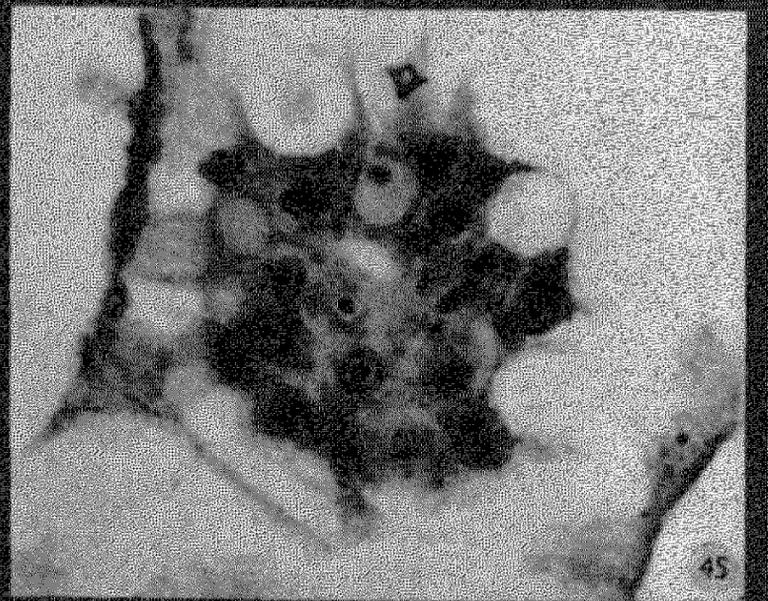
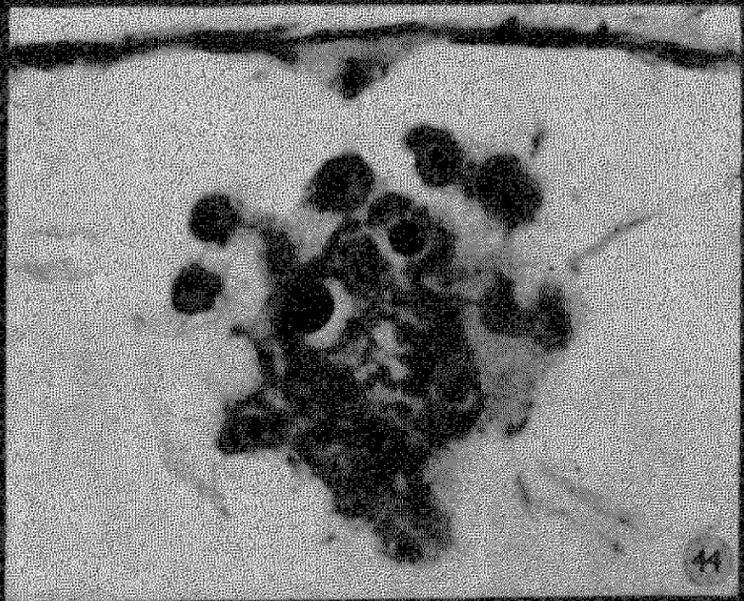
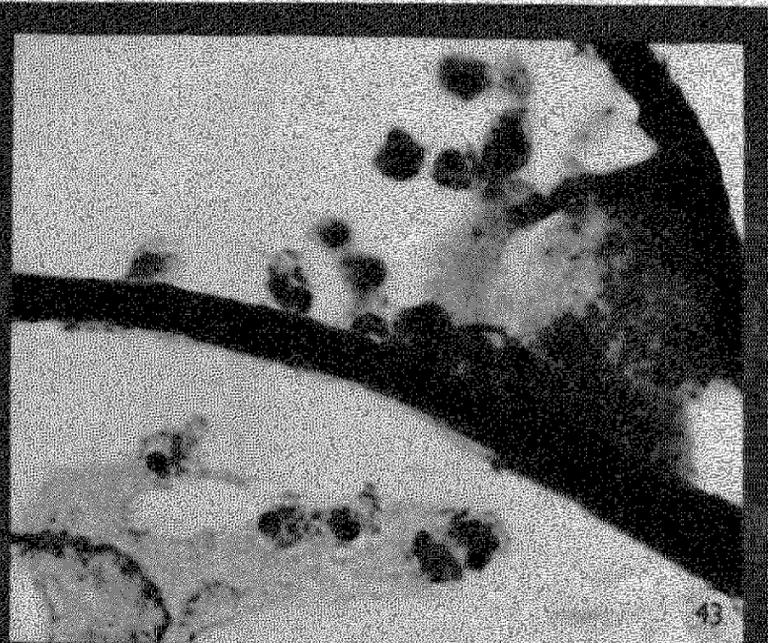
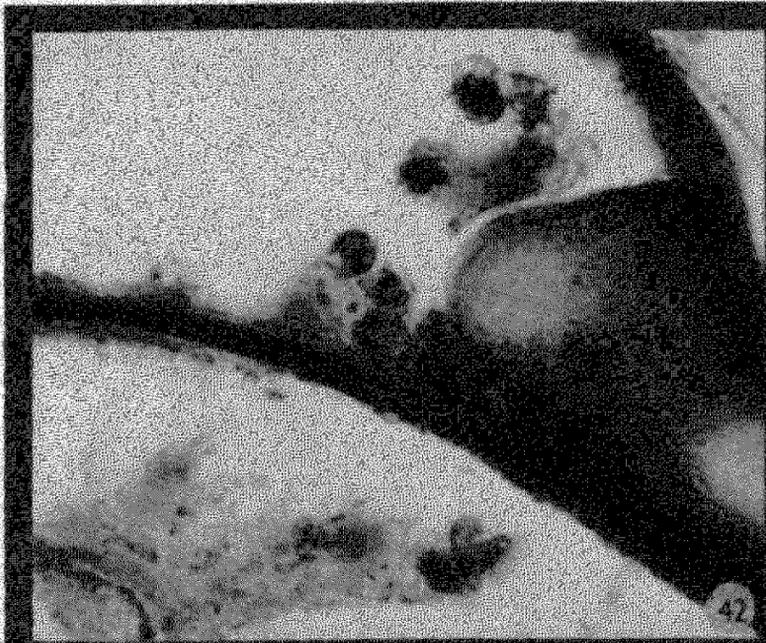


PLATE IV

- Fig. 35. Early stage in tuber infection. Section through a lenticel. The fungus has already reached the centre of the encircled spot. Stained with Heidenhain's haematoxylin. $\times 27$.
Jong stadium van knolaantasting. Doorsnede van een lenticel. De zwam heeft reeds het midden van het omringde gedeelte bereikt.
- Figs. 36, 37. High magnification of the plasmodium present in the encircled spot of fig. 35. Both figures are of the same plasmodium, but are focussed at different levels. Stained with Heidenhain's haematoxylin. $\times 1172$.
Sterke vergroting van het plasmodium in het omringde gedeelte van fig. 35. Beide foto's zijn van hetzelfde plasmodium, maar bij verschillende instellingen gefotografeerd.
- Fig. 38. Stage in tuber infection at which the strands of necrotic cells already extend into the cortex. Stained with Heidenhain's haematoxylin. $\times 27$.
Stadium van knolaantasting, waarbij de strengen van necrotische cellen reeds in de schors doordringen.
- Fig. 39. Part of a necrotic strand surrounded by infected cells. Stained with Heidenhain's haematoxylin. $\times 68$.
Deel van een necrotische streng, omringd door aangetaste cellen.
- Figs. 40, 41. Bodies of unknown structure and origin, present in the cells of the necrotic strands. Stained with Heidenhain's haematoxylin. $\times 1172$.
Lichaampjes van onbekende herkomst in de cellen van de necrotische strengen.

PLATE V

- Figs. 42, 43. The terminal cell of a necrotic strand. In fig. 42 amoebae are present in the necrotic cell and in fig. 43 also in the adjacent cell. Both figures are of the same object but are focussed at different levels. Stained with Heidenhain's haematoxylin. $\times 1172$.
Cel aan het uiteinde van een necrotische streng. In fig. 42 bevinden zich amoeben in de necrotische cel en in fig. 43 ook in de aangrenzende cel. Beide foto's zijn van het zelfde object, maar bij verschillende instellingen gefotografeerd.
- Fig. 44. Amoebae around the nucleus of an infected cell. Stained with Heidenhain's haematoxylin. $\times 1172$.
Amoeben rondom de kern van een aangetaste cel.
- Fig. 45. Promitotic divisions of the nuclei in a young plasmodium. Stained with Heidenhain's haematoxylin. $\times 1172$.
Promitotische delingen van de kernen in een jong plasmodium.
- Fig. 46. Plasmodium around the nucleus of an infected cell. Stained with Heidenhain's haematoxylin. $\times 1172$.
Plasmodium rondom de kern van een aangetaste cel.
- Fig. 47. Plasmodium around the nucleus of an infected cell. Unstained. Phase contrast. $\times 1172$.
Plasmodium rondom de kern van een aangetaste cel.



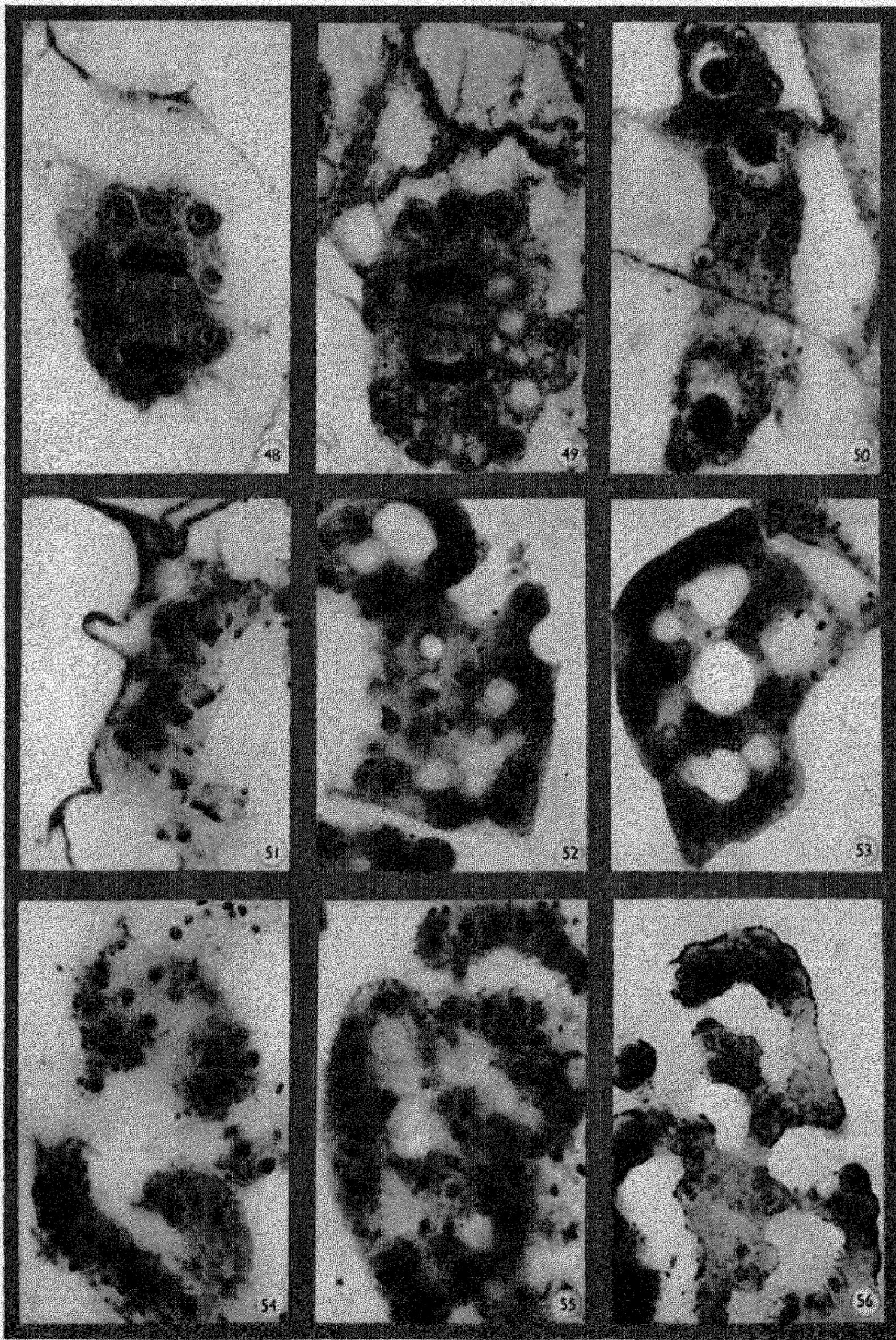


PLATE VI

Figs.48,49,50. Several stages in the division of the host nuclei in the infected cells. Stained with Heidenhain's haematoxylin. × 1172.

Verskillende delingsstadia van de gastheerkernen in aangetaste cellen.

Figs.51,52,53. Meta-, ana- and telophase respectively, of the first mitotic division before resting spore formation. Stained with Heidenhain's haematoxylin. × 1172.

Achtereenvolgens meta-, ana- en telophase van de eerste mitotische deling voorafgaande aan de vorming van de rustsporen.

Figs. 54, 55. Metaphase of the second (Fig. 54) and the third (Fig. 55) mitotic division before resting spore formation. Stained with Heidenhain's haematoxylin. × 1172.

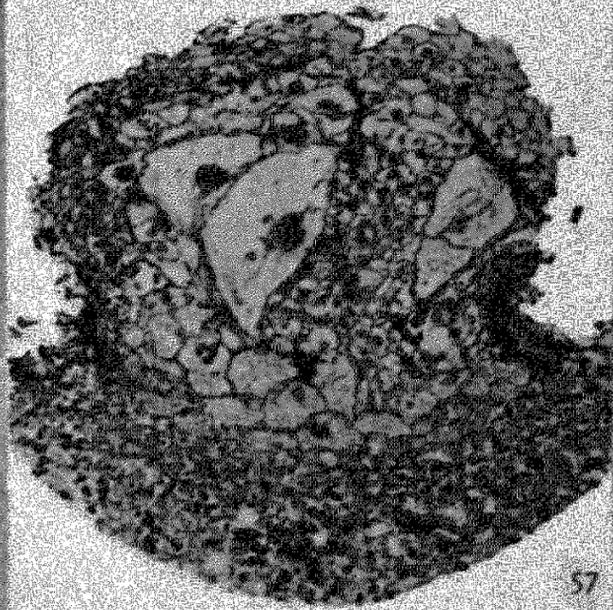
Metaphase van achtereenvolgens de tweede en de derde mitotische deling voorafgaande aan de vorming van de rustsporen.

Fig. 56. Section of a maturing sporeball. Stained with Heidenhain's haematoxylin. × 1172.

Doorsnede van een afrijpende sporenbal.

PLATE VII

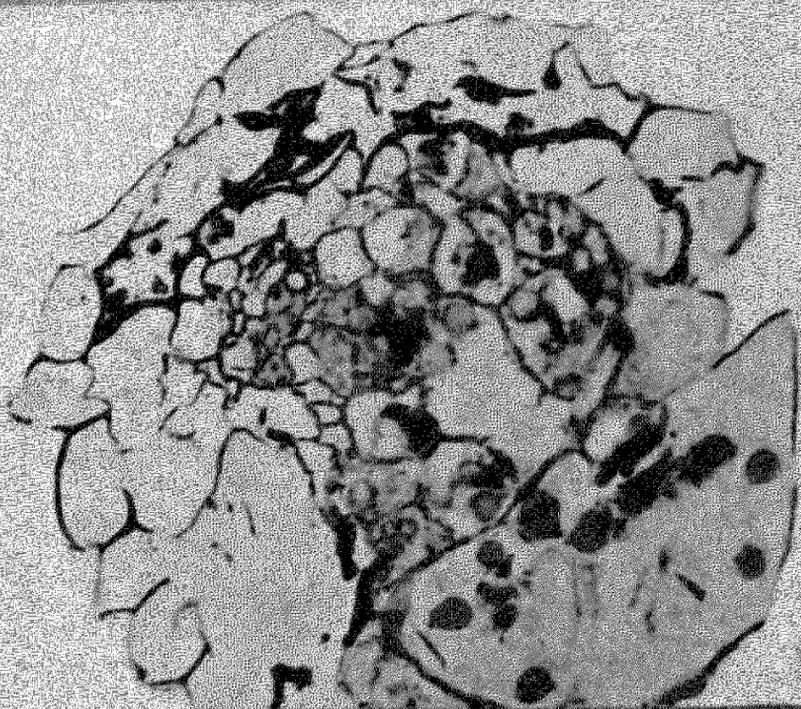
- Fig. 57. Section of a small wart on a potato tuber. Note the giant cells in the wart. Stained with Heidenhain's haematoxylin. $\times 40$.
Doorsnede van een kleine wrat op een aardappelknol. Reuzencellen in de wrat.
- Fig. 58. Section of a big wart on a potato tuber. Stained with Heidenhain's haematoxylin. $\times 13$.
Doorsnede van een grote wrat op een aardappelknol.
- Fig. 59. Young stage in root infection. Necrotic cells, a giant cell, and amoebae present in the infected cells. Stained with Heidenhain's haematoxylin. $\times 320$.
Jong stadium van wortelaantasting. Necrotische cellen, een reuzencel, en amoeben in de aangetaste cellen.
- Fig. 60. Wart development on a root. Stained with Heidenhain's haematoxylin. $\times 20$.
Wratontwikkeling aan een wortel.
- Fig. 61. Wart development on a stolon. Stained with Heidenhain's haematoxylin. $\times 20$.
Wratontwikkeling aan een stolon.
- Fig. 62. Cross section of a mature wart on a root with numerous sporeballs in the wart. Stained with Heidenhain's haematoxylin. $\times 13$.
Dwarsdoorsnede van een rijpe wrat aan een wortel. Talrijke sporeballen in de wrat.



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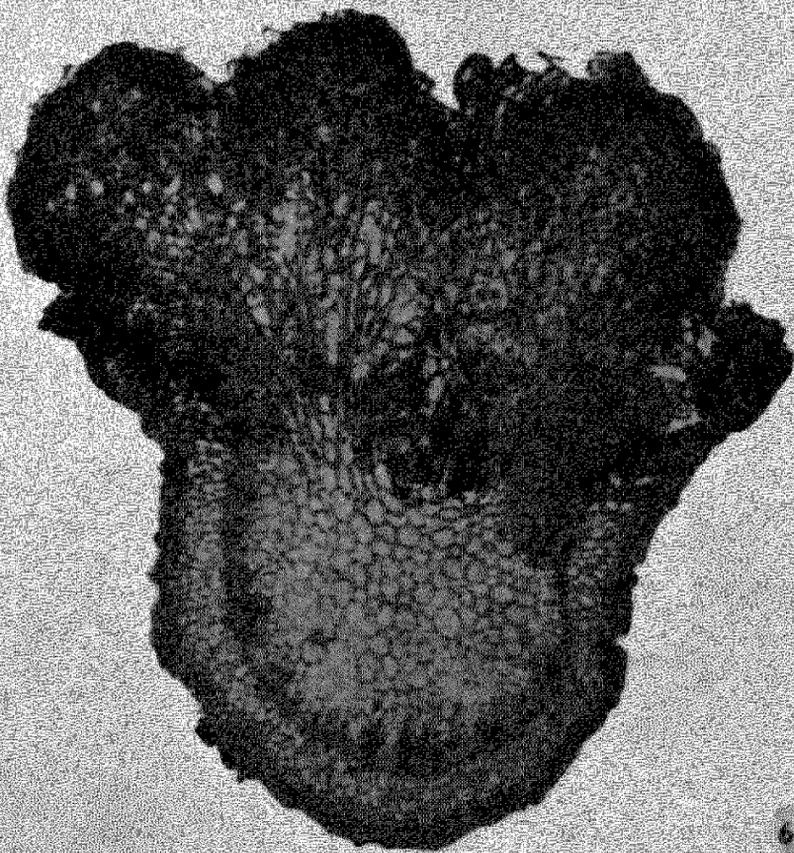
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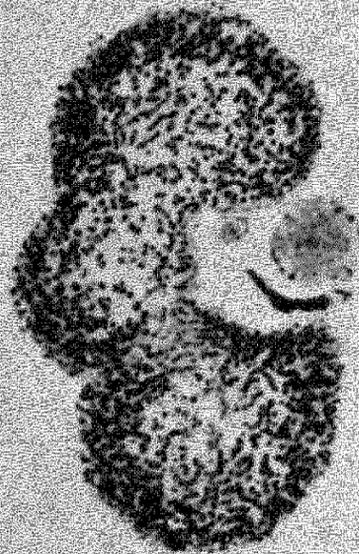
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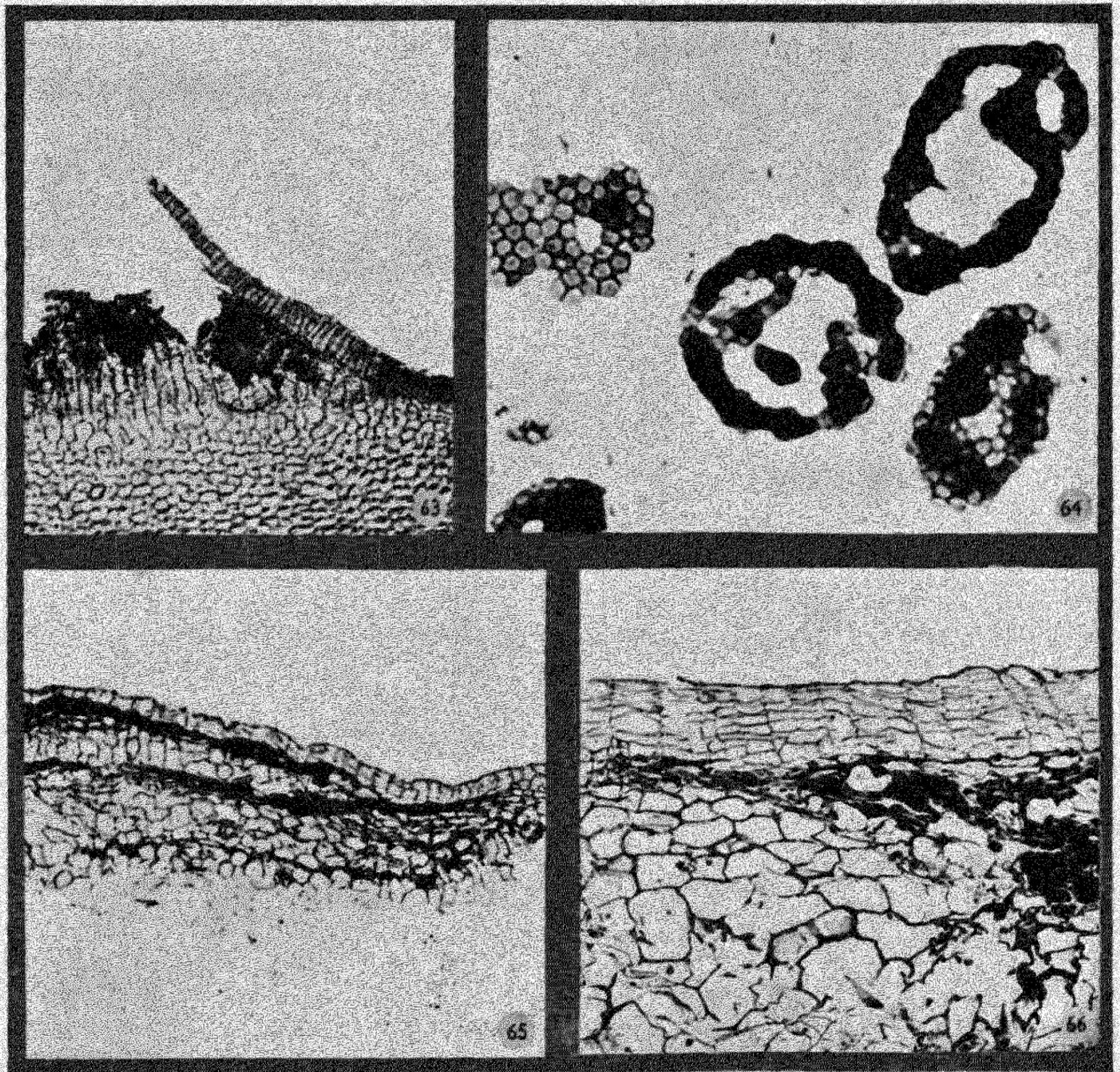


PLATE VIII

- Fig. 63. Section through a powdery scab lesion with remnants of the wart tissue and a woundperiderm underneath. Stained with Sudan III. $\times 13$.
Doorsnede van een poederschurftplekje met overblijfselen van het aangetaste weefsel en wondkurk onder de aangetaste plek.
- Fig. 64. Sections of mature sporeballs in a wart on a root. Stained with Heidenhain's haematoxylin. $\times 540$.
Doorsnede van rijpe sporenballen in een wrat aan een wortel.
- Fig. 65. Subperidermal spread of the infection after lifting. Stained with Heidenhain's haematoxylin. $\times 20$.
Subperidermale uitbreiding van de aantasting na het rooien.
- Fig. 66. Young stage in subperidermal spread of the infection after lifting. On the right there are sporeballs already present in the infected cells and on the left the fungus is spreading in the tissue just under the skin. Stained with Heidenhain's haematoxylin. $\times 60$.
Jong stadium van subperidermale uitbreiding van de aantasting na het rooien. Rechts bevinden zich reeds sporenballen in de aangetaste cellen en links verbreidt de zwam zich in het weefsel vlak onder de schil.