Phytophthora nicotianae var. nicotianae on tomatoes

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STELLINGEN

I

Bij het onderzoek naar de bestrijdingsmogelijkheden van bodempathogenen behorend tot de familie van de *Pythiaceae* is tot nu toe onvoldoende aandacht geschonken aan de rol van de zoösporen bij het ziekteproces in de grond.

II

De conclusie van Ho, dat *Phytophthora megasperma* Drechsler var. *sojae* Hildebrand in de grond hoogstwaarschijnlijk in ziek sojaboonweefsel overblijft, is niet gerechtvaardigd.

Ho, H. H., Mycologia 61 (1969) 835–838.

III

De invloed van de groeiomstandigheden op de lengte-breedte-verhouding van de sporangiën van *Phytophthora palmivora* (Butl.) Butl. is onvoldoende onderzocht om deze eigenschap te gebruiken voor een taxonomische onderverdeling van de schimmel.

Turner, P. D., Trans.Br.mycol. Soc. 43 (1960) 665–672.

IV

Het onderzoek naar de invloed van uitwendige omstandigheden op de groei van groentegewassen onder glas moet meer gericht zijn op de mogelijkheden tot bestrijding van ziekten dan op die tot vermeerdering van de kilogramopbrengst.

V

Bij de bestudering van de invloed van luchttemperatuurwisselingen op de groei van groentegewassen wordt te weinig rekening gehouden met het daardoor veroorzaakte verloop van de grondtemperatuur.

Hussey, G., J. exp. Bot. 16 (1965) 373–385.

VI

Het is verwarrend in de mycologie de term 'vegetatief' te gebruiken, als daaraan een andere betekenis wordt gehecht dan in de botanie.

Ainsworth, G. C., 1967. Dictionary of fungi. CMI, London.
Burnett, J. H., 1967. Fundamentals of mycology. Edw. Arnold (Publ.), London.

VII

Ter bevordering van de internationale communicatie in de fytopathologie dienen gelijksoortige termen afgeleid van eenzelfde stam in de verschillende talen op dezelfde manier gedefinieerd te worden. Dit vereist de instelling van een internationale werkgroep voor de terminologie in de fytopathologie.

VIII
Bij de opstelling van een kostenbegroting voor oogstwerktuigen in de akkerbouw mogen de gevolgen van het te verwachten struktuurbederf van de grond niet buiten beschouwing gelaten worden.

IX
In het Nederlandse Internationale Hulpprogramma moet de opleiding van middelbaar personeel in de ontwikkelingslanden voor diensten in de landbouwsector een belangrijker plaats innemen dan heden het geval is.

X
Niet alleen uit esthetische maar ook uit volksgezondheidsoverwegingen moet het stedelijk openbaar groen zodanig worden ingedeeld, dat gazons en speelplaatsen vrij blijven van hondenfaeces.

XI
De mogelijkheid, die in artikel 1639n van het Burgerlijk Wetboek gegeven is om een dienstbetrekking te beëindigen vóór het verstrijken van de wettelijk vastgestelde proeftijd zonder inachtneming van de voor de opzegging geldende bepalingen, gaat voorbij aan het nut voor alle belanghebbende partijen de reden van de beëindiging te weten.

Contents

I. Introduction

In the last twenty years glasshouse tomato production in the Netherlands increased from 75.000 tons in 1950 to 200.000 tons ten years later and to 379.000 tons in 1970. The acreage increased from 1110 ha in 1950 to 2589 ha in 1960 and 3464 ha in 1965, remaining approximately at that level until the present time (3339 ha in 1970). The rise in production during the firstmentioned decennium was largely obtained by the increase in acreage, but during the second decennium there was a considerable rise in productivity per unit area. This resulted from intensification of production methods, by the construction of glasshouses with better light transmissibility, by improvement of the heating and ventilation systems, of the irrigation and dressing methods and of soil disinfection. Also, the propagation of better plant material concentrated to a greater extent than before on specialised holdings, the rise of temperatures during propagation and after transplanting and extensive application of carbon dioxide contributed to the improvement of productivity.

These changes, especially those concerning propagation and the temperatures used, have had an important influence on the incidence of diseases, such as root and foot rots. These diseases, which have always occurred on tomato in the Netherlands, were known to be caused by the fungi *Rhizoctonia solani* Kühn, *Botrytis cinerea* Pers. ex Fr. and *Didymella lycopersici* Kleb. (Riemens, 1939; Fremouw, 1940; Verhoeff, 1963a and 1963b).

A different type of root and foot rot which was not caused by any of the pathogens mentioned above was found in 1963. Verhoeff and De Mos (1963) considered it to be of a physiological nature. Furthermore, an aberrant type of stembase rot, appearing within a few days after transplanting in June and July, had been reported from 1959 onwards, which was also thought to be a physiogenic disorder (Anonymous, 1959; De Boer, 1960). These two 'physiological' disorders gradually increased in frequency and reached epidemic proportions in 1966 and 1967. It was therefore decided to start a special research project at the Glasshouse Crops Research and Experiment Station at Naaldwijk to investigate these problems. The studies were aiming to find:
a. the cause of the disease;
b. the conditions which enhance its incidence;
c. the ways of prevention or control.

II. The culture of tomatoes

1. In the Netherlands

Several ways of tomato-growing can be distinguished: a. planted in winter in fully heated glasshouses; b. planted in springtime in slightly and non-heated glasshouses and c. planted in summer without heating followed by heating in autumn. The crop in the open air is not mentioned, as this way of growing on a commercial scale has been abandoned in the Netherlands.

   Below, cultivation methods and their history are described briefly, as they may have or have had an influence on the development of the disease reported here. Cultivation can be divided into two separate phases; these are: a. the preplanting period, including preparation of the soil, sowing and propagation of the plant material and b. the planting and post-planting period including all work from planting until the end of the crop, such as planting, management of soil and air conditions, tending of plants, disease and pest control, harvesting etc.

A. Soil disinfection
During the last ten years annual soil disinfection in summer or autumn has become general practice. Previously, steam was used and introduced into the soil through dug-in pipes or chloropicrin was applied. To reduce labour costs, however, pipe steaming was replaced by sheet steaming; besides, methyl bromide became available as soil disinfectant. If root-knot nematodes only are present in the preceding crop, the soil is treated solely with a nematicide. For summer planting alone the soil is not disinfected, because the costs are too high in comparison with the modest revenues of an autumn crop.

B. Sowing and propagation
For small quantities seeds are sown in special sowing compost arranged in sowing boxes and for larger quantities in sowing beds either elevated as benches or at ground level. When the cotyledons are well spread the seedlings are pricked out in potting compost in plastic pots or pressed peat blocks. At present seeds are sometimes sown directly into the pots or blocks. The plants are subsequently placed on the soil of the propagation beds in special glasshouses or glasshouse compartments and kept until ready for planting. This task is executed by the growers themselves or put out to contract. The propagation of plant material on contract by growers specialized in raising transplants was of little importance before the mid-1950's, but developed gradually thereafter. Initially these holdings supplied mainly seedlings for planting in non-heated glasshouses from the middle of March onwards. By the late 1950's they began to supply also plant material for the winter plantings. Consequently, the propagation period at these holdings extended from October till July. Where peat blocks were always used, the top-layer of the propagation beds contained young to-mato roots from November onwards. Disinfection of such beds, before the propagation season started, did not become a general practice until the second half of the 1960's.

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Up till the late 1950's seedlings had been raised at a temperature of 12–14°C at night and 18–20°C in the daytime. Thus, they were usually 11–13 weeks old at planting and had quite lignified vascular bundles. In later periods quick raising at temperatures of 16°C at night and 21°C in the daytime with a maximum of 28°C on very bright days resulted in the use of 7–9 week old plants; these, however, suffered a severe temperature shock at transplanting into non-heated houses.

C. Period of planting
Until the 1950's tomatoes were not planted in heated houses before the middle of January. Improvement of the heating systems and construction of glasshouses with better light transmission, however, allowed planting even as early as mid-December. Interest in summer plantings was small, because plants were raised under flats and subject to the aphid transmitted *Cucumis* virus 1 and *Chrysanthemum* aspermy virus. The complex of each of these viruses with tomato mosaic virus caused severe damage to the growth of the tomato plants. In the second half of the 1950's, however, interest increased again, because by then plants were propagated in glasshouses, protected from aphids.

D. Planting and watering
The seedlings are planted into dibbed holes preferably in soil at 15°C or more. The custom has always been, and still is, to water the plants with a hose pipe immediately after planting, so as to enable the plants to strike roots into the border soil quickly. If subsequent vegetative growth has to be checked in order to further fruiting, watering is usually delayed for some time. This is often practiced when soil- and air temperatures are lower than desirable and the capacity of the heating system is insufficient or non-existing.

Watering is often effected by sprinkling lines which, as long as these lines are suspended at shoulder level, moisten the plants entirely. As soon as the lowest three or four leaves of the plants have been removed, so that water spread will not be impeded, the lines are suspended at approximately 20 cm above the soil. The leaves and stems of the plants then largely remain dry.

E. Temperatures after planting
During the last decade the usual growing temperatures in winter plantings of 15°C at night and 20°C in the daytime were increased to 16°C and 21°C respectively, while allowing a temperature rise to 30°C on bright days. This temperature regime forced the plants into earlier production. The hazardous early non-heated plantings have been replaced by plantings in glasshouses with a hot air or a single pipe heating system. This heating system was aimed to reduce the risks of very early cold plantings but has often tempted the growers successfully to earlier plantings.

On many holdings, glasshouses are often heated until June. The use of the heating systems is then suspended until August or September, because many a grower thinks no heat is required in that period; in this case, however, one forgets that control of the relative humidity of the air is necessary.

F. Pest and disease control
Several measures are taken as a normal routine to control some frequently occurring

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pests and diseases. Soil disinfection, before planting, already mentioned under point A is directed against *Meloidogyne* spp., tomato mosaic virus, *Pyrenochaeta lycopersici* R. Schneider & Gerlach and recently also against *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen. For summer planting also grafting unto resistant rootstocks is applied. Planting holes are often treated with a maneb suspension to reduce infection by *Didymella lycopersici* Kleb. Plant bases are sometimes treated with a suspension of quintozene against *Rhizoctonia solani* Kühn or of thiram against *Botrytis cinerea* Pers. ex Fr.

During the rest of the cropping period regular spraying and/or dusting of pesticides is practiced mainly against *B. cinerea*, *Cladosporium fulvum* Cke and the pests, *Tetranychus urticae* auct. and *Trialeurodes vaporariorum* Westw. A recent development is also the use of predators against these pests. A low relative humidity is desirable to reduce spread of *D. lycopersici*, *C. fulvum* and *B. cinerea*.

G. Duration of the crop

Winter plantings are continued until July and then immediately followed by a summer planting of tomatoes or cucumbers. If the plants are in very good condition the crop may be carried on until August or even October. Plantings in March and later are all ended in the autumn.

2. In Southern England and Guernsey

When the disease discussed in this paper became a problem in the Netherlands it did not in Southern England and Guernsey. It is of interest to try to find the reason for this (see chapter X), but for this purpose it is necessary to point out the main differences in tomato growing techniques by that time between these parts of the United Kingdom and the Netherlands (cf. Anonymous, 1964; Sheard, 1971).

Planting material was propagated by the growers themselves and at lower temperatures than in the Netherlands, i.e. at 14–15°C at night and 17–18°C in the daytime raising to a maximum of 24°C on bright days. Planting was not started until the beginning or the middle of January using plants in pots with the first flowers open. When plants had been pricked off into boxes or peat blocks, planting was done earlier on. The better light conditions (Nisen, 1969; Koninklijk Nederlands Meteorologisch Instituut 1970; Harnett, 1971) allowed a quicker growth of the planted seedlings than in the Netherlands, resulting in a first pick by the end of March. The temperature after planting was 17°C at night and 20°C in the daytime with ventilation at 24°C. Growth of the plants was checked by very careful and regular watering using at times appropriate nutrient solution concentrations and by keeping the plants in pots (whalehide) after standing out. Cultivation in non-heated glasshouses was rare in Guernsey, but did occur in Southern England; then, however, planting was done as late as April. Also in that period the plants received more light than in the Netherlands, resulting in a better and less hampered growth.

Finally the soil was disinfected much more carefully than in the Netherlands, using dug-in pipes for steaming. The regularly used troughs also allowed for a careful disinfection.

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III. The disease

For purpose of identification and comparison with similar tomato diseases abroad a description is given here of symptoms and isolated pathogens.

1. Symptoms
During the course of the work the disease symptoms described below, were found to be caused by the same fungus. Initially, however, some were considered to be of different origins.

A. Foot and root rot
The rot starts as a brown discoloration of the lateral roots or the tip of the tap root. The former easily break and rot away completely, probably by secondary invasion of saprophytic micro-organisms. Usually the infection progresses into the tap root. In young plants the infection of the tap root progresses upwards along the tissue of the vascular bundles as well as through that of the gradually widening pith. At this stage the foliage of the plants takes on a dull dark-green colour; during sunny hours some wilt may show up. Also, adventitious roots are formed at the stem base. When the fungus reaches the stem base, the cortex is infected, which discolours from green to dark-grey. The vascular bundles brown as in the roots and the pith tissue becomes greyish-brown and desiccates. As young plants do not yet possess a complete cylinder of lignified xylem tissue, the desiccation of the pith causes the whole stem base to shrivel. The plants then collapse and die quickly (Fig. 1).

This symptom is mostly found on non-heated commercial holdings within 5–6 weeks after planting in springtime. Less frequently similarly diseased plants are found in crops planted in heated glasshouses in winter.

In older plants a tap root infection often progresses only a little upwards. This may result in a range of foliage symptoms: from darkly discoloured top leaves and yellow basal leaves, when the greater part of the tap root is infected, to only slightly dark-green top leaves, when a small part of the tap root is infected. In the latter case the plants usually start to form a large number of lateral roots from the healthy part of the tap root and may resume normal growth.

When planting is effected in June and especially in July, frequently the stem base alone is infected and not the roots. In this case infection starts at soil level and progresses upwards and downwards. The symptoms at the stem base and the consequences for the plants are identical to those already described. This type of infection takes place within one week after planting.

B. Leaf blight
Occasionally parts of leaflets or sometimes complete leaves discolour bluish-green and wilt. This, at times, happens to plants on propagation beds, of which the leaves have temporarily touched the soil, for instance after heavy overhead watering.

Fig. 1. Root and foot rot symptoms (plant at the left: healthy).
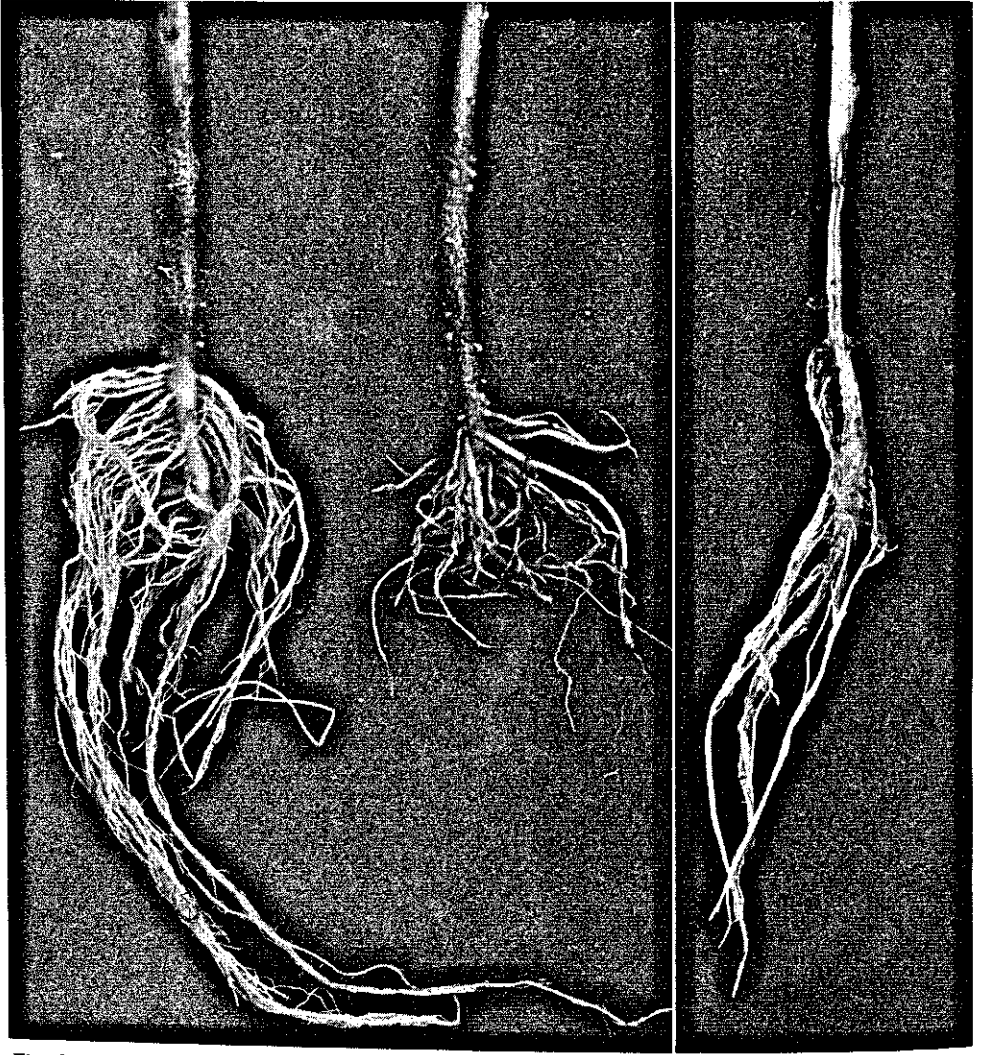
Fig. 1. Wortel- en voetrotsymptomen (links: gezond).

C. Leaf stalk and stem infection

Infected leaf stalks and stems show a blackish-green discoloured lesion. The vascular bundles and the pith tissue have the same symptoms as the corresponding tissues in the stem base, which always include a collapse of the infected parts. Infection of a leaf stalk usually originates from infected leaf blades and may cause infection of the stem, which may also arise from infected fruit stalks or from infection of fresh wounds (e.g. leaf scars). The part of the plant above a stem lesion wilts and in most cases dies quickly, even in older plants (Fig. 2).

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Fig. 2. Stem infection on a young plant.

Fig. 2. Stengelaantasting bij een jonge plant.

D. Fruit rot

The syndrome of this disease also includes a wet fruit rot, usually called 'buckeye rot'. The fruits show greyish-green, partly watersoaked lesions, usually with dark brown concentric rings or parts thereof. Internally, the mucilage turns dark brown to black and decomposes to a watery substance. The fruits maintain their original shape, at times even look somewhat inflated and drop easily (Fig. 3).

In tomato-growing under glass in the Netherlands this symptom mainly occurs on fruits of the lowest truss, sometimes on those of the second lowest. In conformity with leaf stalk infection fruit stalks may also become infected, showing similar symptoms.

2. Closely similar diseases abroad and their respective pathogens

In many other countries symptoms similar to those described above have been attributed to many different *Phytophthora* spp. In 1917 Sherbakoff described buckeye rot caused by *Phytophthora terrestria* Sherbakoff (later corrected in *P. terrestris*). A closely resembling fungus was found in the USA by Reddick (1920) to infect young seedlings (damping-off), the stem of older plants (stem girdling) and the foliage (blight). In Bulgaria *P. nicotianae* v. Breda de Haan var. *nicotianae* was found to cause stem infections, especially on very young plants (damping-off) (Atanasoff and Kovacevski, 1929). In the literature there are many reports of *Phytophthora parasitica* Dast.

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Fig 3. Wet fruit rot, caused by *P. nicotianae*.
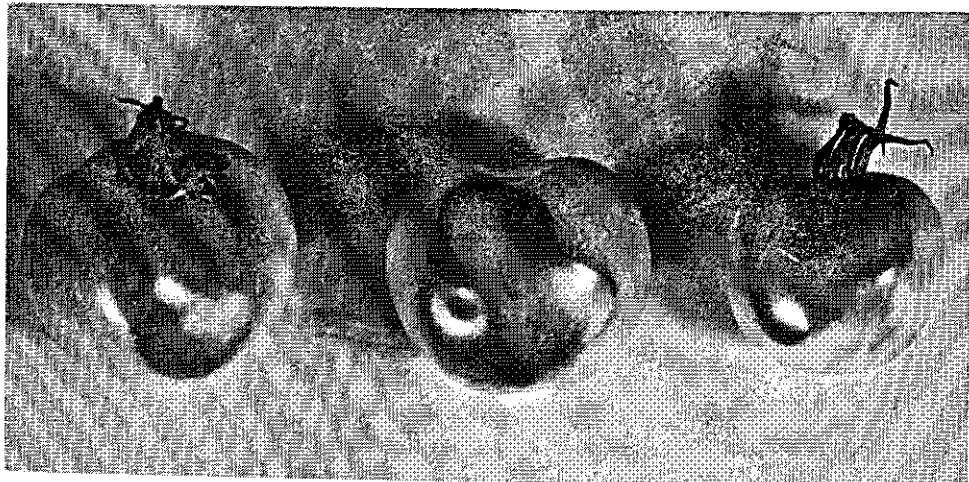
Fig. 3. Nat vruchtrot, veroorzaakt door P. nicotianae.

causing all symptoms described above (for instance: Kendrick, 1923; Richardson, 1941; Williams and Sheard, 1943; Fulton and Fulton, 1951). Foot and stem rot is also caused by *Phytophthora cryptogea* Pethybr. & Laff. (Pethybridge and Lafferty, 1919; Reddick, 1920; Brien, 1940), while Brittlebank and Fish (1927) found this fungus to cause also damping-off. Similar symptoms were provoked in Yougoslavia by an as yet undetermined *Phytophthora* sp. (Aleksić et al., 1969).

A wet fruit rot resembling buckeye rot was caused by *Phytophthora capsici* Leon., according to Kreutzer et al. (1940) and Tompkins and Tucker (1941), while foot rot caused by this fungus was reported by Critopoulos (1955). Tompkins and Tucker (l.c.) also found *Phytophthora drechsleri* Tucker to cause the same type of fruit rot.

Finally there are some incidental reports of other *Phytophthora* spp. infecting tomato plants, i.e. stem rot by *Phytophthora citricola* Sawada (Salerno and Calabretta, 1960); damping-off, root rot and buckeye rot incited by *Phytophthora mexicana* Hotson & Hartge (Hotson and Hartge, 1923); stem infection by *Phytophthora primulae* Tomlinson (Tomlinson, 1952) and by *Phytophthora palmivora* (Butl.) Butl. (Weststeijn, 1964); finally root rot by *Phytophthora verrucosa* Alcock & Foister (Foister, 1940).

Most of the *Phytophthora* spp. mentioned above are considered good and validly published species by Waterhouse (1963). *P. terrestria*, however, she considers to be a synonym of *P. nicotianae* var. *nicotianae*. As to the specific and varietal names 'nicotianae' and 'parasitica' the terminology by Waterhouse is adhered to in this paper, while *P. nicotianae* var. *nicotianae* will be referred to as *P. nicotianae*.

3. Isolations from the Netherlands

As described above, the foot rots as well as the root rots were originally considered to be physiological disorders. In 1964, the Dutch Plant Protection Service isolated a *Phytophthora* sp. from plants with root rot symptoms; at the same time Verhoeff and Weber isolated from similarly diseased material *Phytophthora arecae* (Colem.) Pethybr.

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and a *Phytophthora* sp. closely resembling *Phytophthora richardiae* Buisman (Verhoeff and Weber, 1966). The identifications were made by the Centraalbureau voor Schimmelcultures (C.B.S.), Baarn, the Netherlands. In 1967 and 1968 a *Phytophthora* sp. was many times isolated from all the types of lesions described at the beginning of this chapter. These were identified by the C.B.S. or by the Commonwealth Mycological Institute (C.M.I.) at Kew and found to be *Phytophthora nicotianae* v. Breda de Haan; in 13 out of 15 determinations the fungus was identified in more detail to be *P. nicotianae* var. *nicotianae*. Only once, in January 1971, a *Phytophthora* sp. was isolated, which differed clearly from all former isolates. This isolate formed sporangia very rarely and had a very fluffy aerial mycelium on agar media. The C.B.S. identified the fungus as *P. cryptogea*.

Before 1964 no *Phytophthora* spp. had been isolated in the Netherlands from diseased vegetative parts of tomato plants; on fruits, however, the typical buckeye rot symptoms had already been found around 1920 and *P. nicotianae* had been isolated by the Dutch Plant Protection Service (van Poeteren, 1925). According to communications by growers, fruits with buckeye rot symptoms have been known for long, indicating that this fungus has been widely present in glasshouse soils for many years.

4. Comparison with the disease caused by P. infestans (Mont.) de Bary

In 1968 considerable confusion appeared to exist amongst advisory officers and growers in the Netherlands about the symptoms caused by *P. nicotianae* and *P. infestans*. The fact that *P. infestans* (late blight) had seldom been observed in tomatoes in the past 20 years contributed to this. Moreover, buckeye rot of tomato fruits, caused by *P. nicotianae* had for a long time been called by the growers 'aardappelziekte' (potato disease), a name which usually designates late blight.

In the first edition of the 'Gids voor ziekten- en onkruidbestrijding in de tuinbouw' (Manual for the control of diseases and pests in horticulture, Anonymous 1968) certain symptoms described for *P. infestans* on fruits and stems conform rather to those caused by *P. nicotianae*.

A differentiation between these two diseases is therefore desirable.

A. Symptoms
In comparison with the wet rotten fruits infected by *P. nicotianae* those infected by *P. infestans* show small brown lesions, which often coalesce and the tissue dries out quickly and shrinks. The fruit surface gets lumpy and the fruits themselves less heavy. They remain hanging on the plants for a much longer time than fruits infected by *P. nicotianae* (Fig. 4).

The prevailing opinion amongst advisory officers was, that fruits infected by *P. nicotianae* should show clearly the dark brown concentric rings (buckeye symptom) and that wet rotten fruits without such rings were infected by *P. infestans*. However, fruits infected by the former fungus do not always exhibit these rings.

In order to compare the symptoms under standardized conditions green fruits cv. 'Moneymaker' were inoculated by attaching laterally a piece of mycelium of *P. infestans* or *P. nicotianae* growing on rye agar and on pea dextrose agar with 10 ppm oxytetracyclin, respectively (see chapter IV). The most distinct differences in symptoms were:

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Fig. 4. Dry fruit rot, caused by *P. infestans.*

Fig. 4. Droog vruchtrot veroorzaakt door P. infestans.

	P.nicotianae	*P.infestans*
infected tissue:	soft and turgescent	hard and drying
on wounded fruits:	large brown lesions	small brown lesions
on unwounded fruits:	endocarp and seeds discoloured	only exocarp brown
around calix:	usually infected	usually not infected
vascular bundles near hilum:	usually discoloured in early stage	only discoloured when tissue around calix infected
reddening of fruits:	slowly	very quickly
appearance of epidermis:	green, watersoaked	bronzing

The clearest differences in fruit symptoms are the consistency of the infected tissue, the fact that fruits infected by *P.nicotianae* are mainly found on the lowest truss, while those infected by *P.infestans* may be present on all trusses, and the fact, that the former drop soon when severely infected, contrary to the latter.

Fruit stalks, leaf stalks and stems of the host plant are often infected by *P. infestans*, but rather rarely by *P. nicotianae*. Whilst *P. nicotianae* penetrates into the pith, which consequently becomes hollow and shrivelled, *P. infestans* only infects superficially, the pith remaining healthy, in which case the infected parts maintain their original shape (Fig. 5 and 6). Usually the lesions caused by *P. infestans* are black in colour, but sometimes they colour light brown, especially when formed in summertime.

As the pith and the vascular bundles are not infected by *P. infestans*, the part of the

Fig. 5. Stem infection by *P.nicotianae*.

Fig. 5. Stengelaantasting door P.nicotianae.
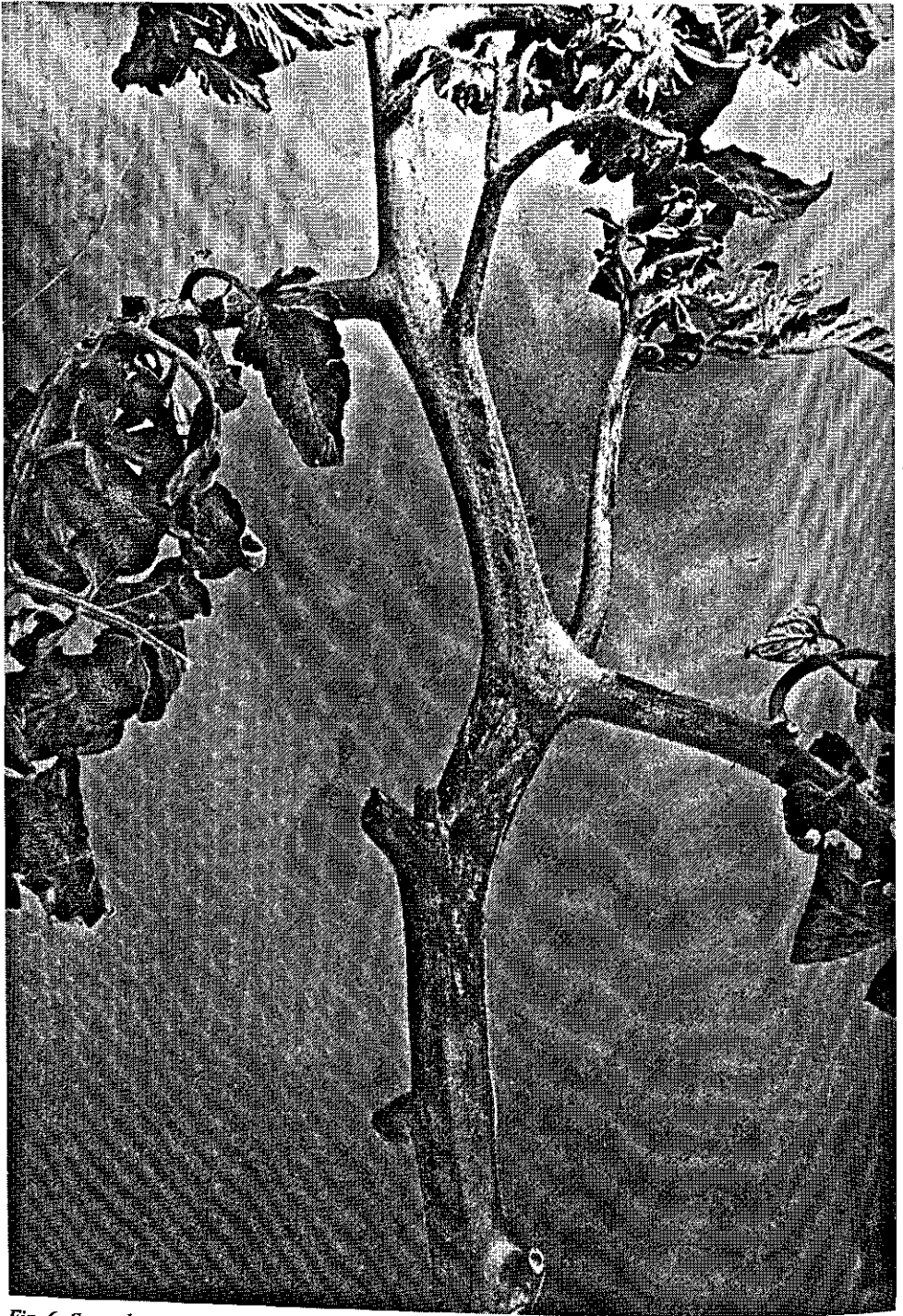
Fig. 6. Stem infection by *P. infestans.*

Fig. 6. Stengelaantasting door P. infestans.

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plant above the lesion does not wilt and recovery of growth remains possible.

Leaf blades become infected much more severely by *P. infestans* than by *P. nicotianae*. Foliage infection by the former may concern a single leaf but often includes many leaves. The leaf blades develop bluish-green lesions and wilt abruptly. When drying they turn light brown.

Contrary to *P. nicotianae* no infection of the stem base or the roots by *P. infestans* has been found. Moreover, artificial inoculation of the roots by this fungus was unsuccessful.

B. Epidemiological aspects

Infection by *P. infestans* mainly takes place, when the fungus has developed abundantly on the outdoor host, the potato, and when the relative humidity also inside the glasshouses is high. Thus, *P. infestans* is found only in dull and moist summers from early June till October. As the source of infection for *P. nicotianae* is the soil inside the glasshouse, infection by this fungus may occur whenever climatic conditions inside only are suitable.

The most obvious entry for *P. infestans* into the glasshouses are open ventilators. Therefore, the first infected plants are usually found straight under these windows, from where some spread to neighbouring plants occurs. Infection by *P. nicotianae* may be found scattered in the glasshouses. *P. infestans* has not been observed on plants in the propagation stage.

When infection by *P. infestans* takes place, tall aerial parts, leaves, stems and fruits, may fall victim, whereas *P. nicotianae* occurs above ground mainly on the fruits of the lowest truss and occasionally higher on some leaves or the stem.

While *P. nicotianae* persists mainly in the soil of the glasshouses (Reddick, 1920; Richardson, 1941), *P. infestans* overwinters mainly in potato tubers (Van der Zaag, 1956), so that the most important source of infection for late blight in tomatoes under glass is the potato crop in the field. Sometimes a storage heap or a cull-pile of potatoes may act as such. This view is supported not only by the period of occurrence of the disease, but also by the observation, that late blight in glasshouse tomatoes hardly occurred in 1968 in that part of the South-Holland glasshouse district, where open fields are no longer available for planting to arable crops.

5. Discussion

Comparison of the symptoms provoked by the *Phytophthora* spp. mentioned in III, section 2 and 3, on several parts of the tomato plant shows a striking similarity. Thus, this plant is not only a suitable host to several species of this genus, but also reacts to them in largely the same way.

The real difference between the tomato pathogens *P. parasitica*, frequently mentioned in the literature, and *P. nicotianae* var. *nicotianae* may be doubted. As will be shown in chapter IV the tomato isolate of *P. nicotianae* var. *nicotianae* from the Netherlands does not infect well developed tobacco stems. Consequently this isolate would have been identified as *P. parasitica* var. *parasitica* according to Tucker's table. From this can be concluded that *P. nicotianae* var. *nicotianae* sensu Waterhouse is not completely synonymous with *P. parasitica* var. *nicotianae* sensu Tucker, but comprises a wider variety of isolates. This is also confirmed by the fact that *P. terrestris* was

considered identical with *P. parasitica* by Tucker (1931) but was renamed as *P. nicotianae* var. *nicotianae* by Waterhouse (1963). For other tomato isolates, reported to be *P. parasitica*, cultural studies would have to be made for correct determination.

It is striking that from the four *Phytophthora* species isolated in The Netherlands from diseased tomato tissue *P. arecae* is taxonomically very near to *P. nicotianae* and *P. richardiae* to *P. cryptogea* (Waterhouse, 1963). According to Waterhouse's key *P. arecae* and *P. nicotianae* var. *nicotianae* concur in the absence or near-absence of oospores in single strain cultures, in having amphigynous antheridia and sporangia of a broadly ovoid or nearly spherical shape (length – width ratio = 1.1–1.4) and about the same size (av. 45×37 and 45×36 μm respectively). These species differ, however, in their maximum temperature for mycelial growth which is approximately 35°C for *P. arecae* and 36.5°C for *P. nicotianae*.

Other differences between *P. arecae* and *P. nicotianae* (var. *nicotianae*) mentioned in the descriptions of these fungi by Waterhouse are not sufficiently constant and may only help to confirm the determination secured from the key.

The main difference between these two species used for determination purposes is their upper limit of temperature for mycelial growth, though this differs only 1.5 °C. An error in the determination is therefore possible. Taking into account the difficulties of the identification and the frequent isolations in the Netherlands and abroad, of which the majority yielded *P. nicotianae*, the identification of the 1964/1965 tomato isolates as *P. arecae* is considered to be an error for *P. nicotianae*.

A similar discussion may be given for the comparison of *P. richardiae* and *P. cryptogea*. The main difference between these two fungi is the near-absence of sex-organs in single strain cultures of *P. cryptogea* versus abundant formation of sex-organs in *P. richardiae*. The fungus isolated by Verhoeff in early 1966 agreed morphologically in some respects with *P. cryptogea* (van der Plaats-Niterink, personal communication) but formed oospores in fair numbers in single strain cultures. For this reason it was identified as 'closely resembling *P. richardiae*'. This isolate, however, originated from a holding in the South-Holland glasshouse district, which proved to be the same, from where in January 1971 a *P. cryptogea* isolate was obtained. An isolate like these two has not been obtained from any other site in the Netherlands so far. It is, therefore likely, that the identifications in question concern the same fungus. This fungus is most probably identical to *P. cryptogea* as the identification of the 1971 isolate did not pose any problems. This would also agree with earlier reports from other parts of the world.

Summarizing, foot rot and root rot of tomatoes in the Netherlands are caused by *P. nicotianae* (var. *nicotianae*) and by *P. cryptogea*; of these the former occurs very generally, whereas the latter has so far been encountered on only one holding. Moreover, this happened towards the end of these studies, so that all experiments have been performed with *P. nicotianae* var. *nicotianae*, unless stated otherwise.

From the comparison of the symptoms caused by *P. nicotianae* and *P. infestans* it has emerged that the main differences between the two diseases are the symptoms on the fruits and the stems, the absence of root symptoms caused by *P. infestans* and the distribution patterns through the glasshouses. As the buckeye symptom is not always clearly visible in fruits infected by *P. nicotianae* (var. *nicotianae*), it is better to relate the trivial name of the fruit rots not to the pattern of colours, but to the consistency of the fruit tissue. Therefore, it is suggested to call fruit infection by *P. nicotianae*: wet (*Phytophthora*) fruit rot and that by *P. infestans*: dry (*Phytophthora*) fruit rot.

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IV. The pathogen

1. Studies in vitro

A. Culture media
It is highly desirable to dispose of culture media, on which the fungus grows readily. Many reports on the nutritional requirements of *Phytophthora* spp. exist in the literature. *P. nicotianae* grows well on natural media like oat meal agar, as do many other *Phytophthora* spp., but develops poorly on synthetic media.

Working with *P. cactorum, P. nicotianae* and *P. erythroseptica*, Lopatecki and Newton (1956) found that glucose and sucrose were adequate carbon sources for growth, the optimal concentrations being 4%. They found the trace-elements Fe, Zn, Mg and Cu to be essential as well as the vitamin thiamin. Other B-vitamins like nicotinic acid, pyridoxin and biotin had little influence on growth (Cameron, 1966).

P. nicotianae grew well on ammonium-N as well as on nitrate-N, though on organic N (alanine and asparagine) growth was even better (Lopatecki and Newton, l.c.). Hendrix and Apple (1964) found considerable stimulation of hyphal growth and sporangium formation, when ether extracts of oat meal, lima beans or cotton seeds were added to glucose peptone agar or when the glucose was replaced by some vegetable oils as the sole C-source. In later studies Hendrix (1965) found cholesterol to improve mycelium development, sporangium formation and zoospore production, especially with *P. nicotianae*.

From this literature survey it can be concluded that growth of the fungus on natural media has not been surpassed by growth on purely synthetic ones. Therefore special attention was paid to supplemented natural media, that would allow a quick mycelial growth of the pathogen and reduce growth of both fungal and bacterial contaminants. This was of importance for isolation purposes.

In order to introduce inoculum into soils, a thick mycelial mat is desirable. For the sake of rapid microscopical identification, translucency of the medium and quick formation of sporangia are needed. Finally, ease of preparation, especially when the medium has to be prepared frequently and in large quantities, is a factor of importance.

In order to compare basal natural media, the mycelial growth of the pathogen was determined in vitro on potato dextrose agar (PDA, Oxoid CM 139), cassava dextrose agar (CDA) and pea dextrose agar (PeaDA). Cassava dextrose agar was made up according to the method described by Weststeijn and Okafor (1971) using 100 g cassava powder, prepared from the cassava selection 53101 of the Nigerian Federal Department of Agricultural Research, which supported good mycelial growth and induced abundant sporangium formation with *P. palmivora* (Hislop and Park, 1962; Weststeijn and Okafor, 1971).

Pea dextrose agar was prepared as follows: 160 g split peas (trade mark Silvo) were soaked in water for at least one hour, boiled until soft, subsequently crashed in a Waring blendor and filtered through cheese-cloth. The filtrate was supplemented with 15 g d(+)glucose and 15 g agar and brought up to 1000 ml with distilled water.

All media were steam sterilized for 15 minutes at 121°C. The quantity of medium

Table 1. Influence of pimaricin and streptomycin on the growth of *P.nicotianae* on different natural media, expressed as increase of the diameter of the colony in mm (mean of five replicates).

	Unsupplemented	+100 ppm streptomycin	+250 ppm pimaricin	+100 ppm streptomycin and +250 ppm pimaricin
PDA	22.8	16.0	17.8	14.9
CDA	48.4	18.1	37.3	5.6
PeaDA	59.3	17.8	22.1	6.1

Tabel 1. Invloed van pimaricine en streptomycine op de groei van P. nicotianae op verschillende natuurlijke voedingsbodems, aangegeven als toename van de diameter van de kolonie in mm (gemiddelden van vijf herhalingen).

per pair of Petri dishes (⌀ 10 cm) was standardized at 10 ml. Each plate was inoculated in the centre with a circular disk 4 mm in diameter taken from the fringe of a colony on a solid medium. The plates were incubated at 25 °C. Radial mycelial growth was measured and the growth rate determined from two days after inoculation onwards (Table 1). Based on these results PeaDA was chosen as the medium for routine cultures. In practice this was also convenient because peas are more readily available than cassava.

B. Addition of antibiotics

For isolation purposes a selective medium was preferred, which is unsuitable to contaminating bacteria and fungi. Therefore, the growth of the fungus on a medium containing the antifungal antibiotic pimaricin and the antibacterial antibiotic streptomycin was tested. Hansen (1960) reported, that *P.palmivora* did not show any visible reduction in growth on PDA supplemented with 1000 ppm pimaricin, but Eckert and Tsao (1960) defining a medium for isolation of *Phytophthora* spp. found an unacceptable growth reduction at concentrations of more than 100 ppm. Therefore in later reports usually only 100 ppm is recommended (Klemmer and Nakano, 1962; Guardia 1968), when the *Phytophthora* spp. are present as mycelium. Haas (1964), Ocaña and Tsao (1965) and Tsao and Ocaña (1969), however, reported that this concentration is too high to allow germination of spores (chlamydospores, encysted zoospores) and they reduced it to 2–10 ppm. Consequently the influence of pimaricin on mycelial growth of the pathogen was studied, the maximum and minimum concentrations tested being 250 and 40 ppm respectively.

Considering antibacterial antibiotics, by addition of 25, 50 and 100 ppm streptomycin to corn meal agar, Eckert and Tsao (1962) found a reduction of mycelial growth of *P.nicotianae* of 17, 25 and 33 %, respectively. No reduction in growth was caused by 100 ppm vancomycin and by 100 ppm penicillin, whereas at 25 ppm oxytetracyclin growth of *P.nicotianae* was reduced by 94 %. Experimental results on the inhibition of fungal mycelial growth by antibacterial antibiotics, however, are not always identical. Whereas in Eckert and Tsao's experiments mycelial growth of *P.citrophthora* was reduced to 8 % by 100 ppm streptomycin, Muller (1958) found that even at 1000 ppm this antibiotic had no effect on the growth of the same species. This may be explained by racial differences in sensitivity to streptomycin or by an effect of the

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culture (composition of the medium, inoculum concentration, age and growth phase of the mycelium) on the action of the antibiotics as was indicated by Lenert and Hobby (1947). Our experiments in vitro point at the second possibility indicating an interaction between culture and antibiotic. Pimaricin and streptomycin-sulphate were added aseptically separately and in combination to a concentration of 250 and 100 ppm, respectively, to the basal natural media PDA, CDA and PeaDA when the media had cooled down to hand-heat. Each treatment was replicated five times. The relative increase in diameter of the colony during a period of four days is given in Table 1.

The results show that on the poorer medium the antibiotics reduced the growth of the fungus less than on the richer media; there is also an interaction between the antibiotics and the media as well as between the two antibiotics. Growth on the supplemented media was too poor to be acceptable, either because of the natural medium (in the case of PDA) or because of the antibiotics (in the case of PeaDA and CDA).

In a similar experiment using PeaDA as the natural medium, streptomycin concentrations of 100, 75, 50, 25 gave a growth of 15, 16, 17 and 30% respectively of that of the control (0 ppm) in a period of five days. Though in this experiment the concentration applied was reduced by a factor four, the growth was increased by only a factor two.

Consequently, oxytetracyclin-HCl[1], another wide spectrum antibacterial antibiotic, was tested, combined and not combined with pimaricin. The latter antibiotic was now used at 40 ppm to avoid severe growth reduction. This choice was based on the minimum inhibitory concentration for fungi like *Fusarium oxysporum* and *Botrytis cinerea* being 40 and 25 ppm respectively. Oxytetracyclin was added as an aseptic suspension in sterile water and PDA and PeaDA were used as the basal natural media. Apart from this, the method used was as described previously. The increase of the colony diameter during a period of six days is given in Table 2.

Obviously pimaricin at 40 ppm had little or no inhibitory effect on mycelial growth and oxytetracyclin-HCl, at 20 and 40 ppm, caused only moderate growth reductions. Antibiotic concentrations of 15 ppm oxytetracyclin-HCl and 30 ppm pimaricin did

Table 2. Influence of oxytetracyclin-HCl and pimaricin on the growth of *P. nicotianae* var. *nicotianae* on PDA and PeaDA (increase of diameter of the colony in mm and in % of growth on unsupplemented medium; means of two experiments; four replicates per treatment).

		0–0*	20–0*	40–0*	0–40*	40–40*
PDA	mm	22.7	18.6	15.0	25.0	18.8
	%	100	82	66	110	83
PeaDA	mm	48.3	55.1	49.2	45.6	41.1
	%	100	114	102	94	85

*Concentration of oxytetracyclin-HCl (first figure) and pimaricin (second figure) in ppm.

Tabel 2. Invloed van oxytetracycline-HCl en pimaricine op de groei van P. nicotianae *var. nicotianae op aardappelglucose-agar en erwteglucose-agar (toename van de diameter van de kolonie in mm en in % van de groei op dezelfde media zonder antibiotica; gemiddelden van 2 proeven; 4 herhalingen per behandeling).*

[1] Vendarcine, Royal Netherlands Fermentation Industries, Delft.

Fig. 7. Growth of *P. nicotianae* (–) and *P. cryptogea* (- - -) in vitro at different temperatures (in % of maximal growth).
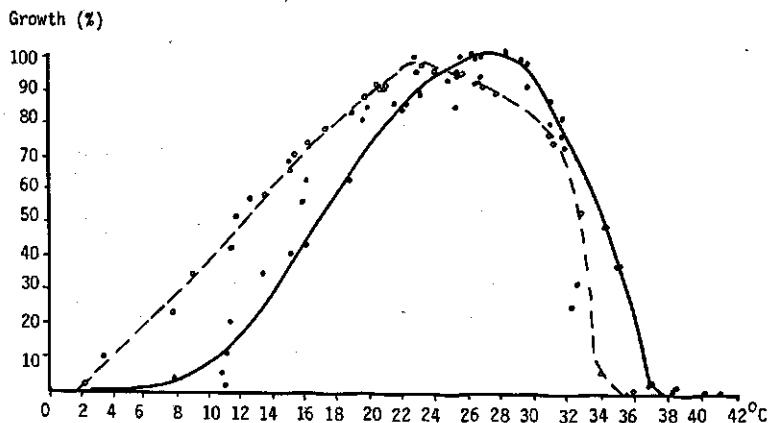
Growth (%)

Fig. 7. Groei van P.nicotianae (–) en P.cryptogea (- - -) in vitro bij verschillende temperaturen (in % van maximale groei).

not retard growth when used either separately or in combination and contaminating bacteria and fungi were kept at a low and thus acceptable level. The use of these concentrations, however, required subculturing within 10 days after isolation, because by then contaminants increased.

Purified isolates were kept on PeaDA.

C. Temperature

The influence of the temperature on mycelial growth was determined in vitro by culturing the fungus on PeaDA and incubating the plates in a serial thermostate. In most experiments light was provided from the side by four 40 W daylight tubes at a distance of about 30 cm from the plates.

All data obtained have been incorporated into one graph (Fig. 7) each point representing the mean of three to six replicates of one treatment in one experiment. From the graph the cardinal temperatures appear to be approximately 6°C, 25–28°C and 37°C. These data agree with those given by Waterhouse (1963). The same series of experiments has been performed with *P. cryptogea*; the cardinal temperatures of this fungus were found to be 2°C, 22–23°C and 36°C (see Fig. 7).

D. Formation of sporangia

Zoospores constitute a convenient inoculum, as the number of zoospores in a suspension can be counted and standardized by dilution. They usually produce only one germ tube each and the germination percentage can be determined. Moreover, zoospores have the advantage of being motile and often of being attracted by exudates of the host plant which, in comparison with aplanospores, enhances their infectivity.

Attempts were therefore made to find a reliable method of producing sporangia and subsequently of zoospores in large quantities. Abundant sporangium production on solid media would be preferable, as this would enable the preparation of a zoospore

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suspension free from sporangia, those of *P. nicotianae* being non-deciduous. Such a method was applied successfully to *P. palmivora* (Weststeijn, 1965). None of the culture media described in the preceding pages satisfied these conditions.

Experiments in vitro showed that changing the concentration of cassava (selection 53101) or pea extract in the medium did not influence the production of sporangia very much. Addition of KNO_3, KH_2PO_4, vitamin B_1 or of different concentrations of glucose (10–20–30 g/l) to these natural media did not give any improvement either.

Chee and Turner (1965) and Hendrix (1965) reported a stimulative effect of cholesterol on the reproduction of *Pythium* and *Phytophthora* spp. Consequently this compound was added to glucose nitrate agar (GNA) at a concentration of 20 mg/l according to the method applied by Hendrix (1965). Light was provided by a 20 W fluorescent tube at a distance of approximately 40 cm from the plates (Hendrix, 1967). The fungus on GNA with cholesterol[1] produced many well-formed sporangia, especially in the aerial mycelium, the fungus on the control medium, on the contrary, produced many submerged and partly plasmolysed sporangia. GNA, however, induced development of a very thin mycelial mat only, but speed of mycelial growth was not affected by cholesterol (cf. also Table 3). When the GNA was replaced by pea dextrose agar or cassava dextrose agar, mycelial growth improved considerably. On both media cholesterol reduced mycelial growth by 20–25% and sporangium production remained poor.

Finally a combination of both substrates was tried with and without cholesterol. The following media were prepared:
1. GNA with 5.4 g glucose per litre according to Hendrix (1965);
2. GNA with 13.5 g glucose per litre;
3, 4 and 5. GNA with 100, 200 and 400 ml pea extract per litre respectively made up from 50 g peas per litre.

Prior to sterilisation the pH of all media was adjusted to 6.5 and after autoclaving at 1 atm for 15 min the pH of the media varied between 6.3 and 6.5. After pouring the plates ($\varnothing$ 10 cm) with 20 ml of medium each, cholesterol was equally distributed over the surface of the solidified media at the rate of 0.4 mg in 0.7 ml of ether per plate. After one night, when the ether was evaporated, the plates were inoculated as usual and incubated at 25°C under the continuous illumination of a 20 W fluorescent tube at a distance of approximately 40 cm.

Mycelial growth was determined over a period of five days and the production of sporangia was assessed 14 days after inoculation. As from the literature was known, that flooding stimulated sporangium production, two cultures of each treatment were subsequently flooded with distilled water and incubated on a laboratory bench at 20–21°C for four days. The number of sporangia was then assessed again. Zoospore liberation was initiated by blowing a horizontal current of air over the open, flooded cultures (Weststeijn, 1965). After approximately 45 minutes this current was stopped and the number of liberated zoospores assessed one hour later. The results are given in Table 3.

The media with pea extract and cholesterol thus seemed applicable for routine sporangium production though the growth reduction brought about by cholesterol

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[1] Brocades, NV. $(\alpha)_D^{20}$ = about –40°; melting point about 148°C.

Table 3. Mycelial growth expressed as increase of diameter of the colony in mm, and production of sporangia and zoospores by *P.nicotianae* as influenced by nutrient medium and cholesterol (means of four replicates; basal medium: glucose nitrate agar, GNA).

	Addition to GNA				
	none	extra glucose	pea extract (ml/l)		
			100	200	400
			– cholesterol		
mycelial growth (mm)	19	19	52	51	65
sporangia:					
before flooding	+++/○	+++/⊙	++/○	++/⊙	+/⊗
after flooding	*	+++/⊙	+++/○	++/⊙	++/⊗
zoospores	*	+	++	++	+
			+ cholesterol		
mycelial growth (mm)	19	16	26	29	41
sporangia:					
before flooding	+++/○	+/○	++/⊗	++/○	+/⊗
after flooding	++++/○	+++/○	+++/⊗	+++/⊗	+++/⊗
zoospores	++	+	+++	+++	++++

*No observation.

Numbers of sporangia or zoospores:
+ = few; ++ = mediocre; +++ = many; ++++ = very many.
Quality of sporangia:
○ = small, empty, plasmolysed or malformed; ⊙ = partly plasmolysed or malformed; ⊗ = well-formed.

Tabel 3. Myceliumgroei, aangegeven als de toename van de diameter van de kolonie in mm, en produktie van sporangia en zoösporen door P.nicotianae onder invloed van voedingsbodem en cholesterol (gemiddelden van vier herhalingen; uitgangsmedium: glucose nitraat agar, GNA).

was a disadvantage. Moreover, good quality sporangia tended to be formed especially on young mycelium.

In order to reduce the risk of contamination, mixing the cholesterol through the medium before autoclaving was tried in accordance with Chee and Turner (1965). The sporangia grown on media supplemented with cholesterol were of better shape and the number of zoospores produced somewhat higher than on the control media, but nevertheless sporangium production in this experiment was not satisfactory. Therefore, a further attempt was made based on the methods applied by Menyonga and Tsao (1966) and by Hine and Aragaki (1963), making use of V8 juice/$CaCO_3$ medium according to Miller (1955). The fungus was cultured in flat-bottom flasks in intermittently aerated liquid medium at a temperature of 25–26°C for 10 days. Subsequently the mycelium was rinsed four times with sterile distilled water and incubated for another 4 days in the same flasks or in large size Petri dishes; it was moistened with sterile distilled water or with 0.01 M KNO_3 (Gooding and Lucas, 1959). The mycelium was then chilled to approximately 12°C and returned to room temperature (approx. 22°C) for zoospore swarming. The resulting suspensions at times contained nearly 10^6 zoospores per ml and could be used for inoculation purposes. If zoospore suspensions had to be kept some hours before being used, they were maintained at approximately 20°C to delay zoospore encystment (Gooding and Lucas, 1959).

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2. Isolation methods

A. Isolation from plant tissue

The pathogen was usually isolated by plating out diseased plant tissue on PeaDA supplemented with 30 ppm pimaricin and 10 or 20 ppm oxytetracyclin. Tender or thin tissue (e.g. rootlets) was rinsed in tap water for a couple of hours and subsequently dried on sterilized filter paper while thick tissue was desinfected with ethanol 95% before plating out. In intermediate cases a disinfectant of intermediate strength was used.

B. Trapping from soil

P. nicotianae could be obtained from soil qualitatively by planting 10 day old seedlings as bait into the soil concerned. When the soil was infested these plants became infected within a couple of days and subsequent isolation from diseased plant tissue was possible. A quantitative estimate of the level of infestation, however, could not be obtained this way.

Green tomato fruits were also used as bait for the pathogen in infested soil. When such fruits (cv. 'Moneymaker') were buried in artificially infested soil diffuse brown, at times dark brown, lesions appeared from both of which the pathogen could be isolated successfully. Wetting this experimental soil to improve formation and motility of zoospores did not increase fruit infection, because green fruits buried in very wet soil became severely infected subepidermally with a white, rod-shaped gramnegative bacterium (the rod length being 2–3 μm), which putrefied the fruits completely. Chilling such soil did not increase infection either and even reduced the speed of lesion enlargement.

In order to assess the sensitivity of this bait technique, soil heavily infested artificially was diluted 2, 4, 8 and 16 times with steamed potting soil. In these mixtures green fruits were buried and after three days they were dug up for lesion counts. The average number of lesions per fruit (ten fruits per dilution) was, starting with the undiluted soil, 8.8, 8.6, 5.1, 4.3 and 2.2, respectively. There was a gradual decrease of the number of lesions per fruit with higher dilutions and for the highest dilution the mean number was too low; moreover in this treatment it varied too greatly, the individual fruit counts being 0–0–0–0–1–1–1–2–6–11. This method was not very sensitive and thus not very suitable, as even at a 16 times dilution the fungal concentration in this soil must have still been high.

Attempts were made to activate the fungus by means of wetting the soil with the following liquids at 400 ml per 3 litres of soil:
a. deionised water;
b. exudate of undamaged green tomato fruits in deionised water;
c. extract of mashed green fruits in deionised water;
d. extract of mashed green leaves in deionised water;
e. 1% glucose solution in deionised water.

Burying green fruits immediately after wetting the soil caused these fruits to rot. Doing so two days after wetting with half of the quantity of liquid (200 ml/3 l of soil), during which period the soil was allowed to dry, gave an average of only 1.4 lesions per fruit after five days, irrespective of the soil dilution mentioned before or of the liquid applied.

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The following modification was found to be a more suitable bait technique. Approximately 75 ml of soil was placed in a tall narrow beaker of 400 ml capacity, filled with approximately 300 ml of a 1% glucose solution and kept to the bottom of the beaker by means of a fine meshed mosquito gauze. This suspension was chilled to approximately 11.5°C, after which green tomato fruits (cv. 'Moneymaker') were floated in it without touching the soil. The fruits remaining in the soil extract were incubated at 20°C to keep the zoospores motile as long as possible (Gooding and Lucas, 1959). After one overnight the fruits were transferred to moist chambers and incubated for another three or four days. As the lesions were then so numerous that they could not be counted individually the percentage of infected fruit surface was assessed.

With this method the rate of infestation of three soil dilutions was assessed i.e. undiluted, 4 times and 16 times diluted. The average percentage of diseased submerged fruit surface, having four replicates per treatment, was 85, 53 and 98, respectively.

This shows that severe infection can occur even in 16 times diluted soil, but also that one or more factors causing considerable variation are playing a role.

When 1% glucose was compared with the liquids mentioned above, the following mean percentages of diseased submerged fruit surface were found:
a. deionised water: approx. 55%
b. fruit exudate: approx. 75%
c. fruit extract: none
d. leaf extract: none
e. 1% glucose: approx. 80%
These figures show, that the influence of the liquid used to inundate the soil was considerable; the absence of infection after inundation with extracts is most remarkable.

3. Pathogenicity

In order to establish the influence of different plant tissues on pathogenicity a series of five isolates of *P. nicotianae* from leaves, stems and roots of tomato was used to inoculate ten green fruits of cv. 'Moneydor' each, half of which were wounded prior to inoculation. Within one week green-brown lesions, often with irregular concentric rings, had been formed on 85% of the unwounded and 95% of the wounded fruits. Another series of seven isolates, originating from stems and fruits, gave 100% infection on wounded as well as unwounded fruits. Roots of 14 day old seedlings of cv. 'Moneydor' were inoculated with the same isolates by means of a 30 minutes soak in a comminuted culture on PeaDA + 10 ppm oxytetracyclin. The seedlings were subsequently planted in healthy potting soil. All isolates infected 100% of the inoculated plants except one isolate, originating from a diseased fruit, which infected only 80% of the plants.

Because *P. nicotianae* is known as the cause of black shank disease of tobacco, the pathogenicity of the tomato pathogen towards tobacco plants was tested. The following *Nicotiana* spp./vars., which were available from work on tomato mosaic virus, were used: *N.tabacum* L. var. 'Xanthi-nc'., *N.tabacum* L. var. 'White Burley-mosaic', *N.tabacum* L. var. 'Samsun-nn'., *N.clevelandii* Gray, *N.rustica* L. and *N.glutinosa* L. In a first series heavily infested soil was planted with four week old seedlings and in a second series six week old seedlings were used. The percentages of plants infected at the end of the experiments are given in Table 4.

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Table 4. Infection of seedlings of *Nicotiana* spp. by a tomato isolate of *P. nicotianae* var. *nicotianae* and influence of seedling age thereon (in % of 24 inoculated plants).

Host plant	Age of plants at inoculation	
	4 weeks	6 weeks
N.tabacum var. Xanthi-nc.	0	0
N. tabacum var. White Burley mosaic	62	0
N. tabacum var. Samsun-nn.	0	0
N. clevelandii	100	100
N. rustica	58	–
N. glutinosa	100	50
Lycopersicum esculentum (control)	100	100

Tabel 4. Infectie van zaailingen van Nicotiana spp. door een tomate-isolaat van P.nicotianae var. nicotianae en de invloed van de leeftijd van de zaailingen daarop (in % van 24 geïnoculeerde planten).

These results show the potential pathogenicity of the tomato isolate to *Nicotiana* spp., a range of susceptibility levels in the genus and a reduction of the susceptibility with increasing age of the plants. In addition, two month old tobacco plants of cultivars susceptible to both physiologic races of the black shank pathogen ('Burley 21' and 'Virginia Gold'), of moderately resistant cultivars ('Burley 37', 'Hicks Broadleaf', 'Coker 111' and 'Coker 316') and of lines resistant to race 0 but susceptible to race 1 (number L8 and its hybrid 'Burley 21' × L8), were planted in heavily infested soil. None of the test plants became infected except 4 out of 50 plants of cv. 'Hick's Broadleaf', while 94% of the control plants (tomato, cv. 'Jupiter') succumbed to the disease. The stems of the same cultivars were wound-inoculated at an age of about 3.5 months, but no infection occurred.

4. Discussion

The influence of antibiotics on fungal mycelial growth may vary considerably with isolates, as well as with culture media. The data in Table 1 show clearly the interaction between culture media and antibiotics. Lenert and Hobby (l.c.) also mentioned the age and the density of the culture as important factors affecting this sensitivity. These factors did not differ initially but may have become increasingly different in the course of the experiment reported in this table.

The mycelial growth, sporangium formation and zoospore production of *P. nicotianae* was much better on V8 juice/$CaCO_3$ medium than on amended or unamended GNA. Consequently the first mentioned medium was preferred for massive production of zoospores. The mycelium formed on this medium contained strikingly dense protoplasm.

Trapping the fungus in soil by burying green fruits was not a sufficiently sensitive method, probably because the fungus stays in the soil in a quite immobile state (mycelium, chlamydospores) and does not grow far from its nutrient base (Mehrota and Tiwari, 1967). Thus contact of the pathogen with the bait might have been limited. This was greatly improved when the fungus was enabled to form mobile spores, as in the floating bait technique. The lack of infection when the soil was flooded with ex-

tracts of leaves or fruits is most remarkable. This result indicates that tomato leaves and fruits contain substances which either inhibit formation or indirect germination of sporangia, or induce immediate and complete zoospore encystment or inhibit zoospore germination. A reduction in formation of sporangia by *P. infestans* after crushing of potato leaves was found by Grümmer and Hahn (1967); a similar type of reaction may be operative with tomatoes.

As to pathogenicity, it is interesting to note that Bell and Haasis (1967) report the infection of tobacco and petunia plants by a *P. nicotianae* isolate originating from *Buxus sempervirens*, whereas Alfieri and Miller (1971) found an isolate of the same fungus from *Zygocactus truncatus* to be pathogenic to tomato and petunia. Ravisé (1970) discussing pathogenicity of eight tropical *Phytophthora* spp. reports, that *P. nicotianae* infects tomato as well as tobacco. However, an isolate from *Bougainvillea* was pathogenic to tomato, but not to petunia (Alfieri, 1970). These and other similar observations indicate the existence of physiological specialization within *P. nicotianae*, which had already been reported in 1962 by Haasis and by Apple.

As the tomato isolate used in these tests did not infect well developed tobacco stems, it should be classified, according to Tucker, as *P. parasitica*. This leads us to suggest, that the isolates of *P. nicotianae* var. *nicotianae* from tomatoes in the Netherlands may not be really different from *P. parasitica* isolates described by other authors as a pathogen of tomatoes.

V. Persistence of the pathogen in soil

Because crop rotation might be a practical measure for the control of *Phytophthora* root rot, it is important to know the period of persistence of the pathogen in the soil.

1. Literature

For *P.capsici*, one of the causal agents of tomato foot rot, Critopoulos (1955) as well as Satour and Butler (1967) found a maximal duration of survival in moist soil of five months. For *P. nicotianae*, however, the latter authors found a period of at least nine months in moist as well as in air-dry soil, their finding being limited by the length of their experiment. Longer periods of survival are reported, amongst others, by Clayton (1958) for *P. nicotianae* under field conditions (5–6 years) and by Zentmyer and Mircetich (1966) for *P. cinnamomi* in moist soil (6 years).

As to the form in which *P. cinnamomi* persists in the soil Zentmyer and Mircetich (1966) have demonstrated survival in invaded organic material either in the form of dormant mycelium or of resting spores (chlamydospores, oospores). They considered the resting spores of lesser importance at least in dry soil (3 % moisture), because under those conditions they could not isolate the fungus from the soil three months after infestation. Ho (1969) deemed it probable that *P. megasperma* var. *sojae* also persists in soil in the remains of diseased tissue. The shorter longevity of *P. capsici* in moist and dry soil in comparison with *P. nicotianae* may be caused, according to Satour and Butler (1967), by the inability of this fungus to form chlamydospores (cf. also Leonian, 1922; Frezzi, 1950; Waterhouse, 1963).

P. nicotianae is able to produce abundant chlamydospores in soil (Trujillo and Hine, 1965; Mehrota and Tiwari, 1967; Seghal and Prasad, 1966) as well as in pure culture (Waterhouse, l.c.; Frezzi, l.c.). *P. nicotianae* probably survives in soil for long periods by means of its chlamydospores. This conclusion was also drawn by Ocaña and Tsao (1965) during their attempts to isolate the fungus from the soil using pimaricin-supplemented media (see chapter IV). Apart from some exceptions, survival as chlamydospores and/or oospores is thought to be the rule in *Phytophthora* spp. (Hickman, 1958; Legge, 1953).

For successful perennation it is necessary that the resting spores can germinate in soil. Conflicting reports have been published about the germination of chlamydospores of *P. nicotianae*. Trujillo and Hine (1965) could not induce chlamydospores to germinate in moist soil smears. Tsao and Bricker (1968) and Tsao (1970) on the contrary obtained ample germination in soils; their success depended on the nutritional composition of the soil.

Germination of chlamydospores is enhanced by soil extracts as well as by moist unsupplemented natural soils, by natural soil supplemented with glucose, vegetable juice or excised citrus roots and by sterilized soils. Germination is inhibited by soil supplemented with asparagine or NH_4NO_3 (Tsao and Bricker, 1968). Similar observations were made by Agnihotri and Vaartaja (1967) for *Pythium aphanidermatum*.

Successful perennation also requires the survival of the developing mycelium after

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germination. Important causes of death of micro-organisms in soil are autolysis (Ko and Lockwood, 1970) and heterolysis. Many authors reported quick lysis of *Phytophthora* mycelium in natural, unsterilized, soil (Zentmyer and Mircetich, 1966; Ho, 1969; Tsao, 1970) and no lysis, or hardly any, in sterilized soil, in which case mycelial development is increased.

Not only lower plants, but also the root systems of higher plants have been found to influence the activity of pathogens in the soil. Dukes (1970) for instance found a drastic reduction of the incidence of *P. nicotianae* (var. *nicotianae*) on tobacco (black shank disease) in soils which had been planted for three consecutive years with groundnuts, ryeweeds, Tagetes or left fallow. On the other hand a maize crop kept the fungus very active.

Apart from the living environment in the soil the physical environment also exerts an influence on the persistence of pathogens and on their renewed growth after a period of dormancy. Lacey (1965) found, that *P. infestans* remained viable for a longer time in soils with a high moisture content than in dry soils; Zentmyer and Mircetich (1966) reported for *P. cinnamomi* a period of persistence of six years and less than three months at soil moisture contents of 20–30% and 3%, respectively. A similar effect of soil moisture was found by Klein (1959) for *P. megasperma*. Toxopeus (1933/34), however, concluded from experiments with *P. nicotianae* var. *parasitica*, that low soil moisture contents do not kill but only weaken the fungus. This effect of soil moisture seems to be a general phenomenon; Griffin (1963) reported considerably reduced activity of many fungi in soils with low moisture contents.

Information about the influence of soil temperature on survival of *P. nicotianae* in soil is scarce. Bell and Haasis (1967) reported that isolation of *P. nicotianae* var. *parasitica* from soil was most frequent, when the soil had been stored at the optimal temperature for mycelial growth of the fungus. In the same year Trujillo and Marcley (1967) reported that the survival of the same fungus is drastically reduced by temperatures below 10°C and above 35°C. At 5°C and 0°C the fungus is killed in less than two days and within a few minutes respectively.

2. Experiments

In order to investigate the persistence of *P. nicotianae* in glasshouse soil in the Netherlands a large sample of sandy soil was collected from a holding near 's-Gravenzande in October 1967. On this soil a tomato crop had to be replanted three times in the spring of the same year, because of nearly complete destruction of the seedlings by *Phytophthora* foot rot. One third of this soil sample was artificially infested to increase the inoculum level, a second third was steam sterilized for three hours in a soil sterilizer to be used as a control for reinfestation. The rest was used untreated. The soil lots were kept in plastic bags to maintain moisture and stored in a glasshouse protected from direct sunshine. The ambient temperature during the four years of storage varied approximately from 10°C to 40°C.

At set times a subsample of each lot was planted in duplo with 16 ten to fourteen day old tomato seedlings, cv. 'Moneydor'. The numbers of infected assay-plants were observed until they remained constant. The last test was carried out four years after the start of the experiment after which no soil for further testing remained. The percentages of infection are given in Table 5. This table shows that the pathogen has

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Table 5. Influence of duration of storage of soil infested with *P.nicotianae* on infection of tomato plants (% of plants infected; 32 plants per treatment).

	Time of test									
	Febr. '68	Apr. '68	July '68	Sept. '68	Dec. '68	March '69	Aug. '69	Apr. '70	Oct. '70	Dec. '71
months of storage	4	6	9	11	14	17	22	30	36*	50
inoculated natural soil	78	94	66	75	84	94	97	97	47	14
natural soil	47	88	59	69	63	78	88	56	22	3
steam-sterilized soil	0	0	16	13	0	0	0	0	0	0

*In this test the soils have been diluted with an equal amount of sterilized potting soil attempting to lengthen the series of tests.

Tabel 5. Invloed van de bewaarduur van grond besmet met P. nicotianae op de aantasting van tomateplanten (% aangetaste planten; 32 planten per behandeling).

not lost much of its virulence for at least thirty months. The test after 36 months has given rather low infection percentages, but this may be due, at least partly, to the dilution of the experimental soil (see footnote at Table 5). Therefore, after 50 months undiluted soil was used; in this test a reduced infection level was found. The infection of plants on steam-sterilized soil in July and September 1968 must be ascribed to insufficient isolation from external sources of contamination.

In another experiment the change of inoculum activity in the soil, as influenced by frequent replanting, was studied using the natural and the artificially infested natural soil mentioned above. The planting treatment superimposed on each soil was such

Table 6. The influence of planting frequency at two soil infestation levels on the infection of tomato plants by *P.nicotianae* (% of plants infected; 108 plants per treatment).

Date of		Way of soil infestation	
planting	observation	natural	artificial
23–10	15–11	19[a]	98[c]
	4–12	23[a]	98[c]
	27–12	24[a]	98[c]
29–12	26– 1	55[q]	48[q]
23–10	15–11	13[a]	94[c]
15–11	4–12	20[a]	81[bc]
4–12	27–12	14[a]	47[b]
29–12	26– 1	10[p]	50[q]

Figures marked with a, b or c differ at P<0.05, those marked with p and q differ at P<0.01. (47[b]< 81[bc] at P = 0.06)

Tabel 6. De invloed van plantfrequentie bij twee besmettingsgraden van de grond op de aantasting van tomateplanten door P.nicotianae (% aangetaste planten; 108 planten per behandeling).

that in one treatment plants were replaced every three weeks by a new batch of young plants and in the other they were not. After nine weeks all plants were uprooted and all plots replanted using one batch of seedlings to assess the infection level on all plots. The experiment was performed in six replications using 18 plants per plot. In Table 6 the percentages of infection of the plants in the natural and the artificially infested natural soil are given at each date plants were replaced.

These figures show that the percentage of diseased non-replaced plants does not increase much after the first period of three weeks. In the artificially infested soil the infection of the replanted treatment decreased with time. This phenomenon might be explained as follows: a) the worsening light conditions reduced the infection of the plants during the course of the experiment as this was carried out in the period from October till January, or b) the virulence or inoculum concentration of the fungus was reduced. The first possibility is considered unlikely as such an influence was not found when comparing the water treatments in a large number of control experiments, which were performed at all seasons of the year as shown in Table 7.

With respect to the second possibility it might be suggested a) that the inoculum concentration in the natural soil has been increased by the extensive root system of the non-replaced plants and b) that the inoculum concentration in the artificially infested soil could not be maintained at the original level by the root system of the non-replaced seedlings nor by that of the replaced ones. Virulence and inoculum concentration have not been separated in this experiment.

The results obtained from the experiments reported here show, that the fungus can remain virulent in moist soil in the absence of the host plant for at least four years, though its activity was declining by the fourth year. Very high inoculum levels may decrease to lower levels even in the presence of a host plant.

Table 7. Percentages of infected tomato plants in artificially infested soils, taken from the water control treatments of a number of experiments performed at different times of the year.

Year	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
1967												60
1968		98		100	100	91						
1969		88	100		12	62				92	93	98
1970				90	100			67	100			76
1971	98		90		67	98						

Tabel 7. Percentage aangetaste tomateplanten in kunstmatig besmette grond uit de controlebehandeling van een aantal bestrijdingsproeven, die uitgevoerd zijn in verschillende tijden van het jaar.

VI. Infection during propagation

1. Introduction

In January and February 1968 frequently *Phytophthora* root and foot rot occurred in tomato plants as early as a few days after planting in the glasshouse soil. Assuming the glasshouse soil to be the source of infection, the incubation time would have been as short as two days. In view of the age (4–6 weeks) and the size of the plants such a short period was considered very unlikely. It was thought, therefore, that the plants might have been infected during propagation. Moreover the disease was often found in a row of 6–7 plants on both sides of a path, that is: 12–14 diseased plants standing together. This number of plants exactly fitted into one box of the type generally used for transportation from the propagation site to the planting site. Diseased plants at different holdings often proved to have been propagated by the same propagation specialist. When the plants at such holdings were placed on plastic sheeting covering the propagation beds and appropriate hygienic measures were taken, the infection hardly occurred again. Thus, there were strong indications that plant material may by infected during propagation. Inspection of the plants on the propagation beds often did not reveal any diseased ones, except in a few cases of bad management or of redundant, overaged, but not yet discarded plants.

Therefore, it was desirable to know accurately the source(s) of infection in order to find more acceptable ways of prevention. For this purpose all phases in the propagation of tomato plants (see chapter II, section B.) were examined for the presence of the pathogen on two holdings specializing in propagation of planting material.

As there has never been any indication of seed infection, which would have shown up as a correlation between the incidence of the disease and the origin of the seeds, the possibility of seed borne infection has been discounted.

2. Sources of root and foot infection

Plant parts other than seeds, and soil in direct contact with the propagated plants were sampled and scrutinized in May 1970 for the presence of the pathogen; they were: sowing compost which had been used for five to six subsequent sowings, young seedlings before pricking out and the soil from very moist parts of the propagation beds. The last was separated into a top soil (top layer of 5 cm) and a sub-soil, which was the layer at a depth of 25–35 cm.

A. Sowing compost and seedlings

Fresh sowing and potting compost has been used for most of our experiments and had never been found to be contaminated. Moreover, the incubation time for *P. nicotianae* on very young tomato plants is so short, that planting in contaminated soil would have caused pre- or post-emergence damping-off. This, however, did not occur in the Netherlands, when soil-less compost was used for the propagation of tomatoes. In order to investigate the presence of the pathogen in sowing compost which has been

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used several times, the compost was planted with young tomato seedlings cv. 'Money-dor' as bait: either 20 seedlings, each one planted individually in a pot of 0,6 l, or 16 seedlings, planted jointly in one sowing box containing approximately 2 l of soil, were used. All treatments were laid out in triplicate. No *Phytophthora* sp. could be detected in the sowing compost from either of the holdings.

On the roots of many seedlings little brown lesions were visible at pricking out. Numerous isolations from such lesions were made on PeaDA supplemented with antibiotics (see chapter IV section 2) but in no case were fungi belonging to the genus *Phytophthora* obtained.

B. Soil of propagation beds

The soil sampled from the propagation beds was tested for the presence of the patho-gen in the same way as the sowing compost. Test plants on the top soil from one hold-ing remained healthy, but those on top soil from the other became diseased to the extent of 40 % in the individual plantings in pots and of 65 % in the joint plantings in boxes. Some weeks before sampling the propagation beds of the first holding had been steam sterilized for one hour as a routine operation before starting propagation for the summer plantings. At the second holding no such sterilization had been effect-ed.

The bio-assay of the sub-soil in individual pots did not yield any seedling infection. Using plants in sowing boxes, however, 45% of those on sub-soil from one of the holdings were infected after three weeks. The difference in sensitivity of the two methods can be explained by the fact that the soil in the sowing boxes was much more densely permeated by roots than in the individually planted pots. The percentage of 45, however, is possibly somewhat inflated as one germinating propagule may have infected more than one plant.

3. Build-up of inoculum in the soil

Preparatory to the 1970–1971 propagation season all propagation beds on both holdings surveyed were steam sterilized. In addition on the first holding (I) the propag-ation beds were treated with approximately 1.5 g zineb (65 % w.p.) per m^2 in a watery suspension and on the second (II) the beds were dusted with approximately 12 g zineb dust (8 %) per m^2 each time the beds were prepared for the accommodation of a new set of plants. In both cases the amount of active ingredient applied was approxim-ately 1 g/m^2.

In December 1970 and April 1971, i.e. one and three propagation cycles after the soil sterilization, the top soil was sampled and bioassayed in order to assess a possible build-up of inoculum with time. No infection occurred from the soil of holding I not even four months after the start of the propagation season; however, though the soil of holding II did not cause infection in December it did so in April, thus showing a build-up of inoculum. At that moment the grower had not yet received any complaints from clients about his plant material being infected by *Phytophthora*. Thus the test method seems to be sufficiently sensitive to detect the pathogen before it causes pro-blems in transplants.

4. Incubation period in relation to age of the host

Apart from the sources of infection, it was desirable to know the incubation period for foot rot in relation to age of the host plant, when seedlings in peat blocks are placed on infested propagation beds. In a small scale glasshouse experiment three groups of five plants each, sown on February 12 and 22 and March 1, respectively, and pricked out into peat blocks 10–14 days after sowing, were inoculated on April 3 by putting the base of the blocks into a thin layer of a mycelial suspension of *P. nicotianae*.

After two days the suspension had dried up and the plants were placed on soil. Eighteen days after inoculation two plants of the youngest age group showed stem infection and two others reduced growth. Three days later a third plant had stem infection symptoms. The growth of the plants of the other age groups was not affected, though some root damage was evident at the end of the experiment three months later.

To investigate this aspect further, at intervals of one week four groups of 30 plants each from the same sowing lot were placed on a layer of 10 cm of heavily infested moist potting compost. The first group came into contact with diseased soil one day after pricking out into peat blocks, the last group did so three weeks after that date.

Of the first and the second group two plants each showed infection of the stem base about 3.5 and 2.5 weeks, respectively, after the first contact with the source of infection. These plants died afterwards. Apart from that, and a slight discoloration of the cotyledons of a few plants in the second and the third group, no other above-ground symptoms were observed.

These results show that root infection of the youngest seedlings only proceeds to stem infection.

Table 8. The influence of the age at which tomato plants in peat blocks are brought into first contact with infested soil on the number of plants killed and on the percentage of infected rootfragments at two distances from the stembase (30 plants per treatment).

Period*	Number of plants killed	Distance from stem base			
		5–6 cm		15–16 cm	
		root fragments per plant	% infected	root fragments per plant	% infected
1 day	2	41.6	16.8ª	25.6	16.4ᵏ
8 days	2	53.2	14.4ª	33.0	19.4ᵏ
15 days	0	52.0	18.7ª	25.0	14.4ᵏ
22 days	0	48.4	34.4ᵇ	30.3	20.8ᵏ

*Period between pricking out into peat blocks and placing onto an infested propagation bed. Figures marked by the same letter do not differ significantly.

Tabel 8. Invloed van de leeftijd, waarop tomateplanten in perspotten voor het eerst in contact worden gebracht met besmette grond, op het aantal dode planten en het percentage aangetaste wortelfragmenten op twee afstanden van de stengelvoet (30 planten per behandeling).

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Apart from the symptoms on stems and leaves those on roots were also observed. For this purpose the roots were carefully cleaned at the end of the experiment six weeks after sowing and the rate of infection determined by cutting and observing pieces of 1 cm long from all roots present at 5 and 15 cm below the stem base. The mean number of root pieces per plant and the mean percentages of diseased ones are given in Table 8.

The number of roots on the differently treated plants is very similar, but the observed level of root infection is higher in the last age group.

5. Stem infection through leaves

Several times infection of the stem during propagation has been observed. At such a lesion the stem shrivelled causing the upper part of the plant to die. These symptoms were invariably connected with infection of a leaf stalk and the corresponding leaf blade; the latter had touched infested soil, by bending over of the stem after heavy overhead watering, and had remained in contact with the soil for some hours before erection. This enabled the pathogen to invade the lamina, progressing subsequently through the leaf stalk to the stem when the atmospheric conditions were moist enough to avoid rapid desiccation of the infected leaf tissue. When this tissue dried very quickly the progress of the pathogen was stopped and the stem was not infected.

6. Discussion and conclusions

The top soil of the propagation beds must be considered the most important source of infection for plant material. This explains the good results from the use of plastic sheeting to separate the peat blocks from the soil of the propagation beds. The sowing soil is usually not a source of infection, though used for sowing several times in succession. Furthermore, the brown lesions on the root systems of seedlings at pricking out are not caused by *Phytophthora* spp.

When allowed to develop unchecked the *Phytophthora* population in the top soil of the propagation beds was able to increase. This explains the infection of planting material several months after the start of the propagation season, even though the soil was steam-sterilized before the season started. In view of the results of control reported in chapter IX, wettable powder formulations of some fungicides can also be usefully applied during the season. Dust formulations are less useful because of limited infiltration into the top soil.

The bio-assay method described using young tomato plants appeared adequate to detect the pathogen in the soil, before disease troubles had been encountered in the planting material.

The incubation time for stem infection of very young seedlings when placed in peat blocks on infested soil was found to be about 2.5 weeks in both experiments, though these were conducted in April and July/August, respectively. The usually quicker development of the pathogen in summer may have been offset by the higher inoculum density in the springtime experiment. The period of susceptibility may be extended, when the speed of growth of the host plant is reduced. This may be so with plantings in winter and springtime. The plants of group one in the second experiment showed aerial symptoms at the same time as those of group two, possibly because during the first

week after pricking out root development took place mainly inside the peat blocks. Consequently, the plants of both groups started rooting into the infested soil at approximately the same time.

When plants were older before contacting the pathogen, stem infections ceased to occur. Probably, the tap root and the stem base of these plants had become sufficiently resistant to prevent foot rot. The lateral roots of the last group, however, showed a higher infection level than those of the earlier groups. This may be an artefact, for these roots started growing into the infested soil at a later date and may not have been as badly infected and decomposed at uprooting as those of the preceding groups. Consequently, during cleaning which was necessary for observation, more dead root fragments of the plants of the preceding groups may have been washed away, leading to the observation of a lower level of infection. Moreover, the plants of these groups have had a longer period for root regeneration, so that the total number of root fragments was not found to be reduced (Table 8).

Prevention of stem infection should be possible by avoiding that leaves are touching the soil or that stems are wetted by watersplash from the soil.

VII. Influence of soil temperature and soil moisture

The temperature and the moisture content of the soil are very important for the development of root diseases. As far back as 1922 Hungerford published on the relationship between these soil factors and plant diseases. During the same period these relationships were studied extensively in Wisconsin, by which time the well known Wisconsin tanks had been devised (Jones et al., 1926).

The literature shows that temperature and moisture content of the soil have a profound influence on the physiology of the host as well as on that of the pathogen infecting the roots of the host plant. Attention will be paid to the way these factors influence the growth, sporulation and spread of the parasite, the activity of the microflora in the soil, the growth and susceptibility of the host plant and the host-parasite relationship with special reference to tomato and to root infecting *Phytophthora* spp.

1. Literature

The influence of temperature on mycelial growth and sporulation of *P. nicotianae* in vitro has been discussed in chapter IV. Saprophytic growth of *Phytophthora* spp. in soil has been found to be very limited (Zentmyer and Mircetich, 1966; Mehrota and Tiwari, 1967), but no data are available on the influence of soil temperature and soil moisture on this feature. Extensive studies on the influence of these environmental factors on the sporulation of *P. nicotianae*, causing gummosis in citrus, have been made by Toxopeus (1933/34). He found that most sporangia were formed in well aerated moist soil at temperatures between 24°C and 29°C. The indirect germination of sporangia in vivo appeared to depend on a sudden temperature decrease starting from 19°C and higher or an increase starting from 14°C or below. A sudden temperature decrease was found to occur in the tropics during heavy showers and may occur in glasshouses during watering.

Increased infection of citrus roots by *Phytophthora* spp. after periodic waterlogging was found by Tsao and Garber (1960) and ascribed to the resulting optimum conditions for sporangial production, zoospore discharge and zoospore dissemination (Katsura and Hosomi, 1963).

Apart from sporulation the moisture content of the soil also influences the osmotic value of the soil water and thus the mycelial growth of the pathogen. Sommers et al., (1970) found a significant reduction of growth of *P. megasperma* and *P. nicotianae* when the water potential of the nutrient medium increased. This also appeared to be dependant on the nutrient status of the medium.

All authors referred to above emphasize that the inoculum density of pythiaceous fungi increases more in moist than in drier soil. Usually such an increase causes a higher rate of infection of the host plant (Johnson and Klisiewicz, 1969; Dukes and Apple, 1968; Baker, 1965 and 1971). This was also found valid for the infection of tomato fruits by *P. nicotianae* (Obrero and Aragaki, 1965).

Physical factors also exert an influence on microbial activity in the soil other than that of the parasite. Chen and Griffin (1966) found the highest diversity of micro-

organisms in a very moist soil at a temperature of 30–35°C. Labruyère (1971) report-ed a higher bacterial population in moist than in dry soils.

Studying the behaviour of tomato plants, Abdelhafeez et al. (1971) found that vegetat-ive growth of the plants was hampered by soil temperatures below 17°C and air temperatures below 20°C, but generative growth was not. The same authors report-ed, for these conditions, a reduction of dry weight, while the roots were thicker and less branched when grown at low than at higher soil temperatures. Calvert (1964) also reported an increase of vegetative growth with higher constant air temperatures and a reduction of growth with lower night temperatures. Hussey (1965) stated that tomato plants at a night temperature of 25 °C have a better growth potential in the daytime than those kept at 15°C at night. The optimum temperature for vegetative growth was found by Riethmann (1933) to be 33°C and by Abdel Rahman et al. (1959) to be approximately 25°C. Abdel Rahman and Bierhuizen (1959) and Abdel Rahman et al. (l.c.) found a higher water requirement per gram dry matter produced at higher soil temperatures.

There are several reports about reduction of host plant resistance at high tempera-tures to pathogens. Yarwood (1965a) mentioned the loss of resistance to *Sphaerotheca fuligena* by *Phaseolus vulgaris* L. and *Vigna sinensis* (Torner) Savi after heating to 45–55°C. More germane to the subject of this paper is the experience of McCarter (1967), that tobacco varieties resistant to *P. nicotianae* var. *nicotianae* at soil tempera-tures of 16°C and 20°C, become diseased at 28–30°C. Johnson and Klisiewicz (1969), made similar observations on the reaction of safflower varieties (*Carthamus tinctorius* L.) to *P. drechsleri.*

There is a very clear influence of soil moisture on the vegetative growth of the tomato plant, which is hampered by a shortage of moisture and enhanced when ample water is available (Abdel Rahman and Bierhuizen, 1959; Abdelhafeez and Verkerk, 1969; Klapwijk, 1972). McCarter (1967) found not only improvement of vegetative growth of tobacco plants at soil moisture contents of 50–70% of the moisture holding capacity in comparison with lower contents, but also a tendency towards reduced resistance of normally resistant varieties to *P. nicotianae.*

Disease incidence depends on the disease potential of the host, which is the ability of the host to contract the disease, and on the inoculum potential of the pathogen, which is a function of inoculum density and of effects of the environment on the activity, including the vigour, of each fungus of a given genetic pathogenicity (Baker, 1965). Many reports have been published describing the relation between disease incidence and the environmental soil conditions, without elucidating the nature of the relationship.

The general opinion that the cardinal temperatures of the pathogen correspond to the cardinal values for disease is supported by many examples (Kincaid and Gratz, 1935; Hine and Aragaki, 1963; McCarter, 1967; Johnson and Klisiewicz, 1969). Accor-ding to Tisdale and Kelly (1926) and Montgomery (1954) *P. nicotianae* does not cause much damage to tobacco and tomato respectively at soil temperatures below 20°C.

Yarwood (1965b), however, doubts the general validity of this opinion and consid-ers the evidence for it inadequate. A contrary example is in work by Richardson (1941), who found the highest percentage of damping-off of tomatoes by *P. nicotianae* at 17°C and 22°C, temperatures, which are well below the optimal temperature for mycelial growth of the pathogen.

The genera *Phytophthora* and *Pythium* are closely related to lower aquatic fungi. Formation and indirect germination of the sporangia of these fungi depend largely on moisture conditions. The soil-borne species of these genera cause more infections when the soil moisture content is high (Wager, 1942; Montgomery, 1954; Tsao and Garber, 1960; Kraft and Roberts, 1969). This agrees with the observations made on the infection of tomato plants on propagation beds. For stem infections of tomatoes by *P. nicotianae* or other *Phytophthora* species a high relative humidity is considered favourable (Brien, 1940; Tompkins and Tucker, 1941; Aleksić et al., 1969).

As to the influence of drought, Zimmer and Urie (1967) mentioned increased incidence of root rot of safflower, when the plants were irrigated after moisture stress. The dry soil probably weakened the plants or was responsible for a higher soil temperature, so that irrigation could have caused a temperature shock (the authors do not consider soil temperatures). Moreover, Katznelson et al. (1956) measured an increased liberation of amino acids and of reducing compounds by plant roots which were moistened after a period of drought. Such exudates may stimulate the activity of a pathogen, as shown for *P. cactorum* by McIntosh (1972). Fulton and Fulton (1951) and Wills (1965) reported wilting of tomato and tobacco plants, respectively by *P. nicotianae*, when a dry period followed a wet one. This may have been caused by severe infection which became visible only during dry weather conditions.

2. Materials and methods

In order to investigate the effect of the physical soil factors on the *Phytophthora* root and foot infection of tomatoes, a series of experiments at different soil temperatures and moisture levels was conducted in Wisconsin tanks, which were placed in a greenhouse under natural light conditions. The construction was basically identical to that originally described by Jones et al (1926).

Initially the soil containers were $43 \times 27 \times 25$ cm and suspended in static (non-circulating) water. However, with the air temperature varying between 15 and 20 °C and the water thermostats set at 10 °C and 25 °C there appeared to be a temperature difference between the bottom of the pan and the soil surface of approximately 2 °C and 4 °C respectively. Usually also a temperature gradient in horizontal direction could be measured within each pan.

To reduce the influence of the air temperature on that of the soil, the size and the shape of the containers were modified in such a way that the contact surface between containers and water was considerably enlarged and that between soil and air reduced. To this purpose each Wisconsin tank was provided with a perforated, 5 mm thick pvc cover supporting 72 cylindrical pvc containers each with a depth of 30 cm, an outer diameter of 50 mm and an inner diameter of 46 mm. The water in the tanks touched the lower side of the covers; the soil level in the containers was kept 2–3 cm below the waterlevel (Fig. 8).

The water temperature, measured by means of thermocouples and recorded by a Honeywell recorder type Electronik 16, showed an unacceptable decline from the top to the bottom of the tank. Circulating the water by means of one small centrifugal pump per tank was sufficient to obtain an equal temperature throughout the tank. The construction of the mercury thermostats caused this temperature to fluctuate with

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Fig. 8. Set-up of Wisconsin tanks.
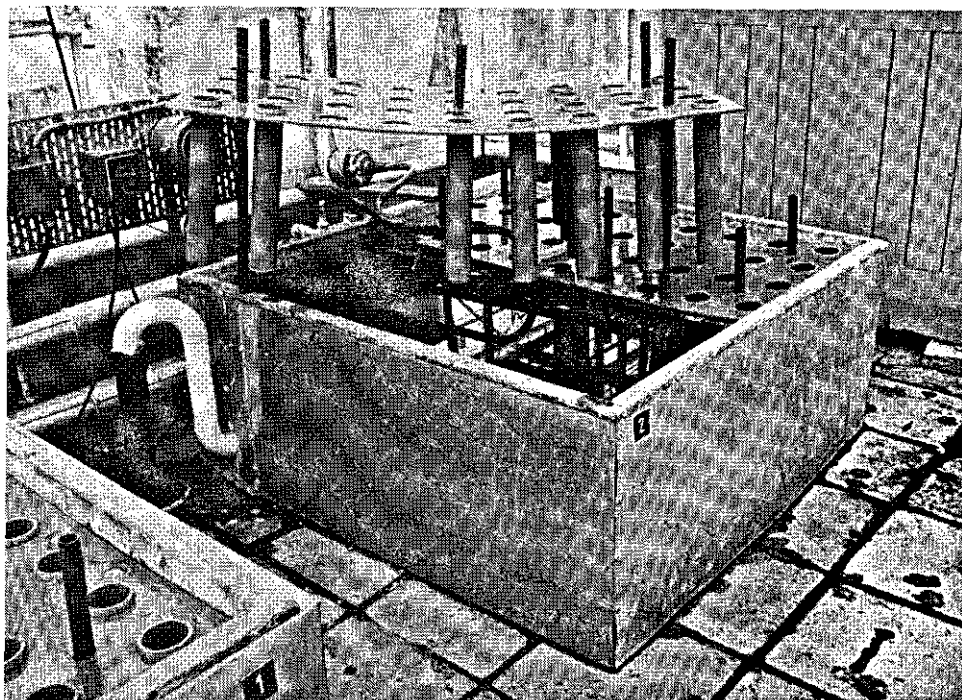
Fig. 8. Inrichting van de Wisconsin tanks.

time by approximately 1.5–2°C (Fig. 9). The soil temperature was measured at 24, 14 and 4 cm below the soil surface and found to follow closely the water temperature as shown in Table 9. In this way the soil temperatures 4 cm below the soil surface could be maintained even when the air temperature was approximately 20°C higher and this was increased later on by quicker circulation of cold water and better heat insulation of the pvc covers and the soil.

Soil moisture studies were conducted by means of the same equipment. Starting from a soil sample with a known, but low, moisture content (determined by weighing and drying) several moisture levels were obtained by addition of water. These levels were maintained by regular weighing and watering of each tube individually.

3. Soil temperature

The tubes described in the previous section were filled with moist potting soil, through which inoculum was mixed at the rate of approximately one plate ($\varnothing$ 10 cm) of *P. nicotianae* on PeaDA per 2 litres of soil. The tubes were placed in Wisconsin tanks at 10, 15, 20 and 25°C respectively. After acclimatisation for 24 hours seven week old tomato seedlings, cv. 'Moneydor' were planted in this soil with a bare root system and subsequently watered from the top with 50 ml of water. Each treatment was duplicated using 72 plants per replicate.

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Fig. 9. The influence of air temperature and of stirring and cooling of the water on the water temperature at different depths in the Wisconsin tanks (thermostat: 10°C).
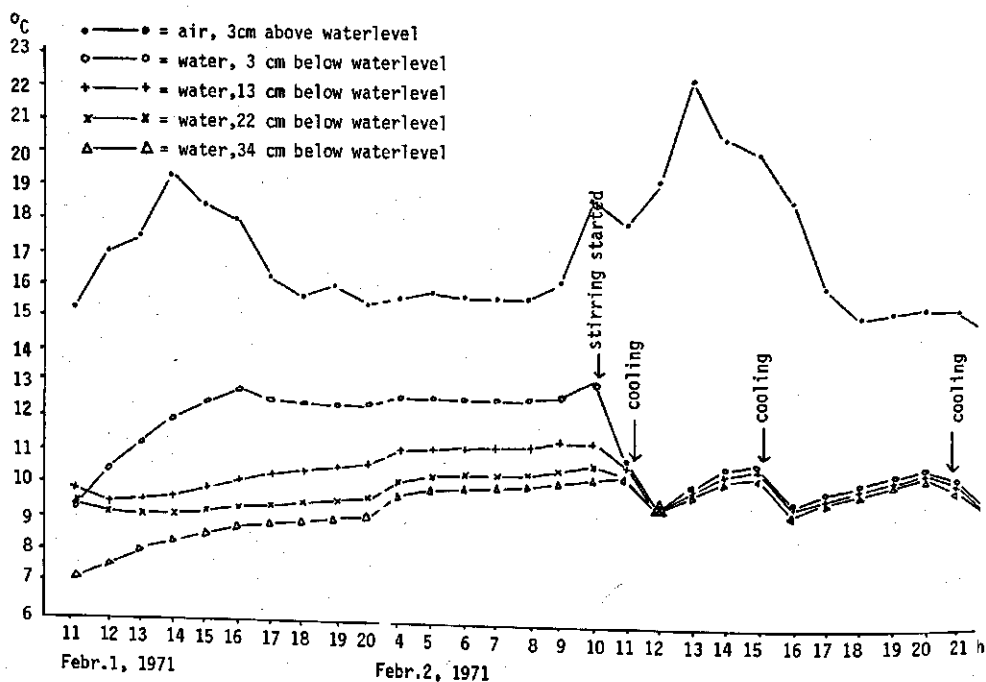
Fig. 9. De invloed van de luchttemperatuur en van rondpompen en koelen van het water op de watertemperatuur op verschillende diepten in de Wisconsin tanks.

Table 9. Temperature just above and in the soil in relation to the set water temperature in Wisconsin tanks and to the glasshouse temperature at various dates.

	Distance from surface (cm)	Observation date and setting of water temperature								
		Febr. 10, 1971		March 7, 1971			April 2, 1971			
		25°C	10°C	25°C	15°C	10°C	25°C	15°C	10°C	
water	−10	24.5	10	25.7	14.7	12	25.5	14	10.2	
soil	−24	24.5	10							
	−14	24.7	10							
	− 4	24.5	10	26	14.7	11.7	25.5	14.2	10.2	
air	+ 3			32	28.2	27.5	25.2	20.2	23.2	
	+40*	20	17	33.5	31.5	32	28	25	27.5	

*Glasshouse temperature

Tabel 9. Temperatuur juist boven en in de grond in relatie tot de ingestelde watertemperatuur in Wisconsin tanks en tot de ruimtetemperatuur op verschillende data.

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Number of plants killed
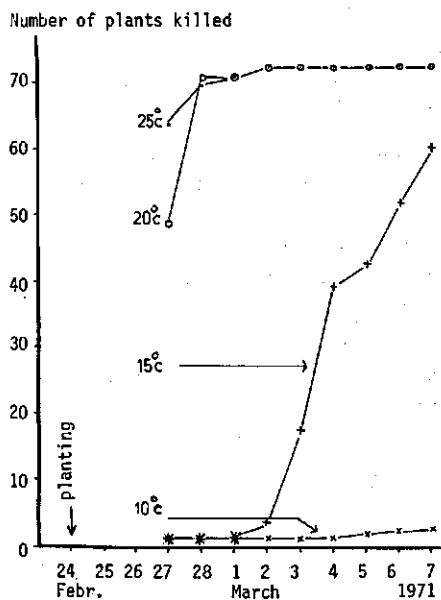
Fig. 10. Influence of soil temperature on the number of plants killed after planting in soil infested with *P. nicotianae* (72 plants per treatment).

Fig. 10. Invloed van de grondtemperatuur op het aantal gedode planten na uitplanten in met P. nicotianae besmette grond (72 planten per behandeling).

Two days after planting wilt symptoms were visible in the 25°C treatment and the next day rapid death of seedlings was observed. At 20°C the plants were killed slightly more slowly. In these two treatments the plants appeared to have been killed by the stem base rot discussed in section 5 of this chapter. Significantly less plants were killed at 15°C and hardly any at 10°C, as shown in Fig. 10. The stem lesions at 25 °C were considerably longer than those at 20°C, the mean lengths being 5.9 and 4.1 cm, respectively. At 15°C few stem lesions occurred and infection nearly always started at the roots. The mean lesion length on tap root and stem at 15°C was 7.1 cm and at 10 °C 1.1 cm. The data for the two higher and the two lower temperatures are not comparable as the former treatments had to be observed at an earlier date due to their sudden collapse.

Such rapid death at higher temperatures was avoided in the following experiments by applying the water into the soil and not on top of it. This application was achieved by passing the water through a tube of 1.5 mm wide introduced into the soil. When under these conditions vigourously growing test plants, especially those in peat blocks, were used little root infection was observed by the end of the experiment. Transplanting one month old seedlings with a small loose ball of disease-free soil gave a low death rate, but significant differences in root infection by the end of the experiment. The root infection was determined for each plant after careful rinsing and rated from 1 = healthy root and stem base to 6 = severe infection of collar of tap root and stem base collapsed. Dead plants were rated into class 7. The root infection index was calculated as the mean of the individual root infection rates. The mean root infection index at 10°C appeared significantly lower than at 15°C ($P < 0.01$) and the latter again lower than those at 20°C and 25°C ($P < 0.05$), which did not differ.

The temperature treatments, however, caused considerable differences in length of

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the epicotyl and number of leaves formed, as shown in Table 10 for one of the temperature experiments. Within each temperature the epicotyl of the control plants was slightly longer than that of the diseased ones (P = 0.06), but the numbers of leaves did not differ.

A temperature experiment was also carried out with two week old seedlings planted with a bare root system into infested soil, which was moistened before planting. The soil temperatures had been raised to 12, 17, 22 and 27°C in order to allow some growth to the plants in the coldest treatment. Water was always applied into the soil and not from the top. The experiment lasted one month and by the end the root infection

Fig. 11. Influence of soil temperatures on number of plants killed after planting in soil infested with *P. nicotianae* (112 plants per treatment).
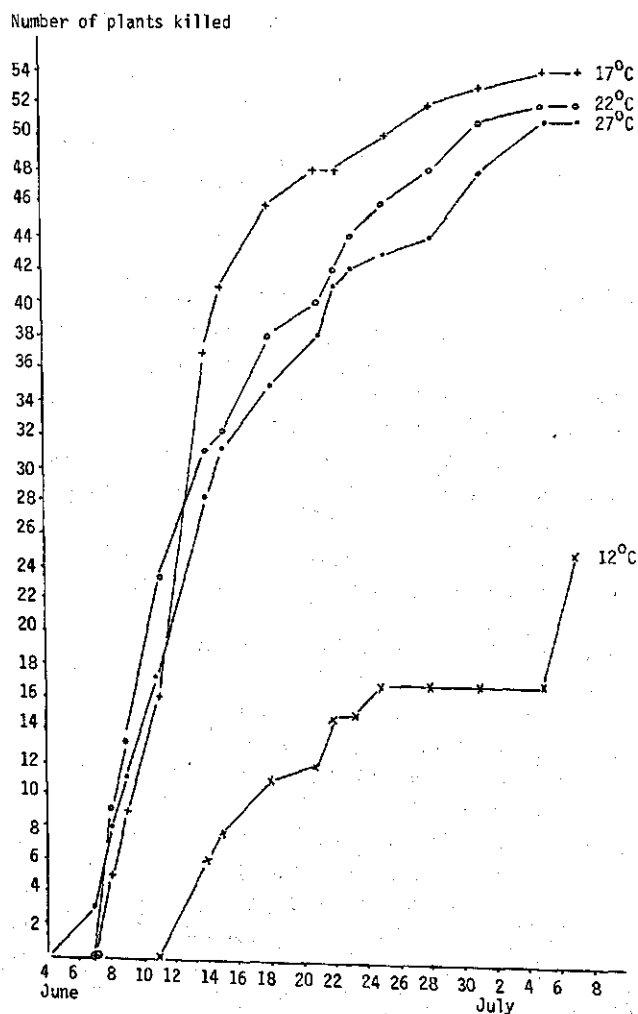

Fig. 11. Invloed van de grondtemperatuur op het aantal gedode planten na uitplanten in met P. nicotianae besmette grond (112 planten per behandeling).

Table 10. Influence of soil temperature on length of epicotyl and number of leaves of symptomless tomato plants in infested and non-infested soil, using 100 and 35 plants per temperature respectively.

Temp.	Length of epicotyl (cm)		Number of leaves	
	soil infested*	control*	soil infested*	control*
10°C	5.2^a	5.6^f	2.5^k	2.5^p
15°C	6.5^b	6.8^g	3.6^l	3.8^q
20°C	7.2^c	7.4^h	4.0^m	4.0^r
25°C	8.5^d	8.9^i	4.3^n	4.2^s

Figures within each column marked with different letters differ significantly at $P<0.05$.
*The control plants were slightly longer than the infested ones ($P = 0.06$), but the numbers of leaves did not differ.

Tabel 10. Invloed van de grondtemperatuur op de lengte van het epicotyl en het aantal bladeren van symptoomloze tomateplanten in besmette en onbesmette grond bij gebruik van 100 resp. 35 planten per temperatuur.

index, including the dead plants, was calculated for each treatment. In the sequence of the temperatures mentioned above the indices were 3.6, 5.1, 4.9 and 5.1. The root infection indices of the living plants only at the end of the experiment were 2.4, 3.1, 3.1 and 3.2, respectively. In both cases the first figure differed significantly from the others ($P < 0.01$). The curves for the number of plants killed during the course of the experiment are given in Fig. 11. This experiment was conducted in June, so that high air temperatures may have stimulated infection. The influence of temperature on vegetative growth of the host plants was of the same order as given in Table 10.

Finally, the influence of soil temperature at and above the optimal temperature for mycelial growth of the pathogen was tested. Seedlings of cv 'Moneydor', in small round peat blocks, were transplanted at an age of five weeks into the tubes of the Wisconsin tanks, which were filled with heavily infested sandy soil. The temperatures were kept at 26, 28.5 and 31°C. At the start the soil had a moisture content of 32%, which decreased by transpiration of the plants and was returned to the original level at regular intervals by weighing and watering. The percentages of plants with diseased stem bases and the root infection indices are given in Table 11.

These data show that soil temperatures above the optimum for mycelial growth of the pathogen are unfavourable for root infection.

Table 11. The influence of high soil temperatures on root infection by *P. nicotianae* (48 plants per treatment).

Soil temp. (°C)	Diseased plants (%) after days*						Root infection index	
	9	16	29	36	45	49	dead plants included	dead plants excluded
26	0	6	19	30	38	38	4.6	2.8
28.5	0	2	2	6	15	26	3.5	2.5
31	0	0	0	0	0	0	2.0	2.0

*Number of days after transplanting into infested soil.

Tabel 11. De invloed van hoge grondtemperaturen op wortelaantasting door P. nicotianae (48 planten per behandeling).

4. Soil moisture

The influence of soil moisture was investigated by planting a one month old healthy seedling, pricked out in a small round peat block, into each of the cylindrical containers previously described containing heavily infested sandy soil with a moisture content of 27 or 37%. The highest moisture content made the soil nearly muddy and the lowest provided a soil which remained crumbly. The experiment also included the soil temperatures of 13–14°C and 25–26°C in order to investigate temperature – moisture interactions. Each treatment consisted of sixteen plants and was replicated four times.

The soil dried out because of water used by the growing plants, but the weight of the tubes and thus the mean moisture content of the soil was restored by addition of water when the soil of the moist and the dryer treatment had desiccated to an average of approximately 25% and 18% soil moisture respectively.

The plants killed during the course of the experiment were dug up and those showing root infection were recorded. The experiment was ended after one month, when all plants were uprooted and the root infection indices determined as described before.

Table 12 shows that the root infection indices for those plants which were still alive at the end of the experiment did not differ, but that those for living and dead plants together showed a higher level at the lower soil moisture content though only at the higher soil temperature ($P < 0.05$). The same trend is found in the number of plants killed during the course of the experiment.

The mass of lateral roots, which was assessed visually at uprooting and coded from 0 (= none) to 5 (= very many), was higher in the moist than in the drier treatment.

Table 12. The influence of soil moisture and soil temperature on root infection, root formation, death rate and transpiration of tomato plants grown in soil infested with *P. nicotianae* (64 plants per treatment).

	Soil temperature (°C)			
	13–14°C		25–26°C	
	Soil moisture		Soil moisture	
	37%	27%	37%	27%
root infection index 1*	2.3ᵃ	2.6ᵃ	2.4ᵃ	3.2ᵃ
root infection index 2*	2.6ᵏ	3.2ᵏ	3.1ᵏ	4.8ˡ
quantity of roots per plant**	4.0ᵖ	3.1�q	4.0ᵖ	2.6�q
number of plants killed	4	10	10	35
transpiration in ml/plant	181ᵗ	113ˢ	418ᵘ	132ˢ

Figures within each row marked with different letters differ significantly at P<0.05.
 *Root infection index 1 refers to the plants alive at the end of the experiment only; root infection index 2 refers to all plants including dead ones.
 **Assessed by coding 0 = none to 5 = very many.

Tabel 12. Invloed van vocht en temperatuur in de grond op wortelaantasting, wortelvorming, afsterving en transpiratie van tomateplanten, geweekt in met P. nicotianae besmette grond (64 planten per behandeling).

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The mean transpiration, also given in Table 12, shows a reduction of activity of the plants by lowering the moisture level and the temperature.

In order to compare the root infection indices of living plants in soils with a normal and a high moisture content at different intervals after planting into infested soil, an experiment was laid out, in which 108 seedlings, comparable to those used in the previous experiment, were planted into infested sandy soil with a mean moisture content of 37% and the same number at a mean moisture content of 22%. Every two or three days the moisture loss was replenished. Eight days after the start of the experiment a batch of 12 plants was uprooted from both treatments and the root infection index determined. At alternating intervals of three and four days this operation was repeated untill all plants had been used up. Observations were also made on the number of plants dying during the experiment because of infection by *P. nicotianae*.

The root infection index of the living plants, that is of all plants scoring a root infection rate of four or lower, as well as the percentage of dead plants at each uprooting operation is given in Table 13. This shows, that there is no difference between the level of root infection of living plants in the moist and the dry soil from twelve days after planting onwards. The low index found for the plants in dry soil eight days after planting is due to lack of root formation, so that insufficient contact has yet been made between the roots and the pathogen. Most plants in the dry soil, however, eventually died, whereas most of those in the moist soil continued to grow. At each uprooting the living plants in the latter treatment had a dry weight 1.5–2 times as high as those in the former, which indicates the difference in growth between the two treatments.

Table 13. Root infection indices of living plants and percentage of dead plants after growth in infested soil at a high and a low moisture content for increasing periods.

	Soil	Number of days after planting								
		8	12	15	19	22	26	29	33	36
Root infection index	dry	1.7	2.2	2.4	3.1	2.7	3.3	3.5	2.7	3.0
	moist	2.9	2.3	3.2	3.0	3.4	3.2	3.0	3.0	3.0
Percentage of dead plants	dry	0	33	42	42	75	58	83	55	75
	moist	8	25	25	17	17	17	9	17	17

Tabel 13. Wortelaantastingsindices van levende planten en percentage dode planten na groei gedurende toenemende perioden in besmette grond met een hoog en een laag vochtgehalte.

5. Soil temperature and moisture in relation to stem base rot

As described in chapter III a stem base infection occurred frequently very soon after planting at high air temperatures in June or July. The collapse of the stem base tissue was ascribed by the growers to a direct heating effect and thus considered to be a physiological disorder. Inoculation experiments, performed in the course of these studies at high temperatures with and without the presence of *P. nicotianae* showed, however, that these symptoms only developed in the presence of the pathogen. Moreover, the pathogen could always be isolated from diseased stem tissue.

From the experiments reported under section 3 of this chapter the soil temperature and the availability of free moisture at the soil surface proved to be of critical importance for this type of stem base infection, i.e. at soil temperatures below 20°C or when free water at the soil surface was lacking, the infection did not take place.

As will be shown in chapter IX, the infection could also be prevented when the plants were watered from the soil surface with water at 35–39°C. The conditions governing the incidence of this type of infection support the hypothesis that stem base rot is caused by zoospores, which are liberated when the sporangia in the soil are subjected to a quick temperature decline by means of free water.

6. Discussion

At soil temperatures between 17°C and 27°C the infection of roots was more severe than at temperatures below and above. This may be due to the decrease of activity of the pathogen in the low temperature zone, but only partly so in the high temperature zone. In the latter case the root infection index of the 31°C treatment was only slightly lower than at 28.5°C and 26°C, so that the reaction of the plant must provide the explanation for the conspicuously lower death rate at that temperature.

A distinct influence of the moisture content of the soil on the activity of the pathogen has not been found, the root infection indices for living plants in dry and in moist soils being largely the same. There was, however, a great influence of moisture on the number of plants that died following infection, this number being higher in the drier soils. In view of the bigger mass of roots in moist soil (see Table 12) it can be concluded that plants in moist soils have a better opportunity than those in dry soils to overcome root infection by formation of new roots, which take over the function of the infected ones. As these plants continue to live and even to grow, they can proceed to a resistant stage. Within the soil moisture limits for vegetative growth of the host plant it seems that the infection-promoting effect of soil temperatures between 17°C and 27°C can be offset by an increase of the soil moisture. A similar interaction was found with *Sorosporium reilianum* (Kühn) McAlpine in sorghum (Christensen, 1926).

A synergistic effect of temperature and moisture is found, when tomato plants, standing in soil at a temperature of 20°C or more, are watered excessively with cool water, so that the stem bases are in the water for a short time. Under such conditions of free moisture and sudden temperature decline zoosporangia are likely to produce zoospores, which infect the stem bases of the plants. This explanation is supported by the experimental result that watering with water of 35–39°C does not bring about this stem base rot.

No indications have been obtained that tomato plants acquire less resistance with age when grown at higher temperatures or that resistance is weakened, as reported for tobacco by McCarter (1967) or for safflower by Johnson and Klisiewicz (1969).

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VIII. Influence of nitrogen and potassium nutrition of the host

1. Introduction

Addition of nutrients may act directly on the pathogen in the soil or indirectly through the nutrition of the host plant.

The literature gives ample evidence for the influence of nutrition on the susceptibility of the host plant to fungal pathogens. Amongst the various elements, nitrogen is the most important and high N-dosages in general increase the susceptibility. Potassium is of lesser importance and usually exerts an opposite influence (Gäumann, 1951). Phosphorus is often mentioned at a third instance, as its effects depend much on the N-and K- levels in the plants (Gäumann, 1951; Krausz, 1969). Other elements come to the fore even less.

For these reasons and because Ten Houten (personal communication) had found a reducing effect of N on the incidence of *P. nicotianae* var. *nicotianae* in tobacco in Indonesia, attention was paid to the influence of N and K on the reaction of tomato plants to the pathogen.

2. Literature

Concerning the direct effect of nutrients in the soil Gilpatrick (1969) reported that retardation of *P. cinnamomi* on avocado by soil amendments with alfalfa is possibly due to NH_4^+ concentrations in that soil of 60–200 ppm, while spores and hyphae of this fungus were killed in vitro at 85 ppm. The inoculum potential of pathogens may also be reduced by increase of the activity of antagonists, for instance by addition of crop residues (Weinhold, 1969). On the other hand, the persistence and the inoculum density of soil borne pathogens may be enhanced by soil amendments (Trujillo, 1969), while addition of nutrients may have a direct influence for instance on spore germination, as indicated in chapter V.

Concerning the indirect effect of nutrition on disease development, the susceptibility of plant tissue to fungal diseases generally increases with unbalanced increase of nitrogen above average (Gäumann, 1951; Böning, 1952). From a literature review on the influence of host nutrition on infection by parasites (Krausz, 1969), fungal infection appeared to be enhanced by a high nitrogen content of the host in nineteen instances, but never by a low nitrogen content. Dressings with calcium cyanamide did not stimulate fungal diseases as this fertilizer has fungicidal properties in the soil. In two cases a high nitrogen concentration as compared with average N-levels, reduced infection, and in three cases a low nitrogen concentration did so.

According to Alten and Orth (1941) and to Klein (1956) nitrogen dressings caused higher concentrations of low molecular sugars and amino acids in the plant tissue and Abdalla and Verkerk (1970) reported higher nitrate contents. The increased damage by *P. infestans* to potatoes which are well provided with nitrogen, is ascribed by some authors (Lepik, 1940; Hagenguth and Griesinger, 1941) to the high content of organic nitrogenous compounds in the plant tissue, which allow rapid growth of the pathogen;

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others, however, found infection by and growth of this pathogen to depend mainly on the carbohydrate content (Grainger, 1962; Birnbaum, 1962). Tobacco appeared to be more susceptible to *P. nicotianae*, when the N-level in the rooting medium (sand) was high (Apple, 1961).

Krausz (1969) quoted no examples of high potassium levels enhancing susceptibility of the host plant, but eleven of low potassium doing so. Infection was not found to be reduced by low potassium levels, but he mentioned seven references to high potassium levels doing so.

Potassium was found to influence the susceptibility of potatoes to *P. infestans* only slightly, which influence depended on the nitrogen nutrition (Weindlmayr, 1965). Kincaid et al (1970) found a moderately reliable quadratic relation between potassium content in the soil and incidence of black shank disease of tobacco. Dukes and Apple (1963) found no correlation between exchangeable K and the inoculum potential of the black shank pathogen in the soil, so that K should exert its influence on the disease incidence through the virulence of the pathogen or the susceptibility of the host.

The above mentioned data show the importance of host nutrition in general for the rate of infection by fungal pathogens. Could a practical method of controling *P. nicotianae* on tomatoes along these lines be found, there would be less need for the use of fungicides. Therefore, a series of experiments was conducted to investigate the influence of nitrogen and potassium on this disease.

3. Materials and methods

The experiments were conducted under glasshouse conditions, using young plants, which were allowed to adapt the chemical composition of their tissue to the nutrients presented to their roots before being inoculated with the pathogen. In the first experiment the nutrients were mixed through a N- and K-deficient basal white peat substratum. This, however, had the disadvantages, that increase of N- and K-salts also increased the total salt content of the substratum and that uniform root inoculation was not possible without serious damage to the root system of the plants. Moreover, observation of the roots was not possible during the experiment and was difficult at the end. Therefore, in subsequent experiments, plants were raised in aerated nutrient solutions of given composition. In this case each plant was grown in a 0.8 litre bucket, in which the nutrient solution was replaced by a fresh quantity, when the liquid was reduced to more than half, or kept at the same level by continuous replenishment and circulation from one storage tank for each treatment of each replicate. These tanks were filled with fresh nutrient solution when needed. This method was a modification of the one described by Uittien and Steiner (1969).

The nutrient solutions were composed according to the method described by Steiner (1961) and the sum of the ionic concentrations standardized at 30 mg ions per litre. Nitrogen was given as NO_3^- and replaced by Cl^- in the decreasing concentration range. K^+ was replaced by Na^+ in the potassium range. The concentration of all other ions was kept constant, while the pH was standardized at 6.0–6.5.

Aerial parts were inoculated by a piece of an agar culture of the pathogen covering it with moist cotton wool and enveloping it with plastic. Roots were inoculated by planting in infested soil or by soaking in a suspension of mycelial fragments and/

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or zoospores, cultured in V8 juice/$CaCO_3$ medium (chapter IV). Chemical analysis of the peat substratum, the nutrient solution and the plant tissue was made by the Soils Department of the Glasshouse Crops Research and Experiment Station at Naaldwijk.

Root infection of young seedlings in the white peat substratum was assessed by calculating the root infection indices as described in chapter VII. Root infection of the plants grown in nutrient solutions was assessed according to the following system of scoring: 1 = no discoloration; 2 = small superficial lesions; 3 = half of the roots with dead portions; 4 = half to three quarters of the root system dead; 5 = more than three quarters of the root system dead. The length of the lesions on stems and leaf stalks was measured and the period between inoculation of the leaf stalk and leaf fall determined.

4. Experiments

A. NK-experiment in white peat

In the first experiment white peat was dressed with 6 kg of a mixture of $CaCO_3$ and $MgCO_3$, 0.5 kg of double superphosphate and 0.5 kg of a trace elements preparation (Sporumix A) per m^3. N and K were added in three concentrations, i.e. 50, 250 and 500 g N/m^3 (applied as ammonium nitrate limestone, 23 % N) and 60, 300 and 600 g K_2O/m^3 (applied as potassium sulphate, 48 % K_2O). The pH was 5.5. Eighty-four one week old seedlings cv 'Moneydor' were planted in the substratum of each of the nine possible N-K-combinations, from which after a pretreatment period of two weeks sixty plants were pricked out each into a 0.6 l pot, filled with identical, but artificially infested soil. The experiment was laid out as a partially balanced lattice design according to Cochran and Cox (1957) with three replicates per treatment and twenty plants per replicate.

At the end of the pretreatment period the length of the lamina of the largest leaf of the first leaf pair was measured to assess the influence of the treatment on growth. This character showed greater differences than plant length and leaf number. The experiment lasted eight weeks, after which the plants were uprooted and the root infection indices determined.

The results are given in Table 14 and show significantly longer leaf blades at the

Table 14. The influence of nitrogen and potassium dressing of a white peat substratum on the growth of tomato plants and the infection of their roots by *P. nicotianae*.

	N (g/m^3)			K_2O (g/m^3)		
	50	250	500	60	300	600
Soil analysis (mg/100 g air dry soil*)	61	187	380	78	297	567
Mean length of leaf blade (mm)**	23	19	18	21	19	19
Root infection index***	6.3	6.3	6.5	6.1	6.6	6.4

N_{50}>N_{250} and N_{500} (P<0.01); K_{60}>K_{300} and K_{600} (P = 0.04) *K_{300}>K_{60} and K_{600} (P = 0.06)
*79 % of the dry matter of the basal white peat substratum was organic.

Tabel 14. De invloed van stikstof- en kalibemesting op de groei van tomateplanten en de wortelaantasting door P. nicotianae.

lowest nitrogen level (P<0.01). The same tendency was found with potassium (P = 0.04). As the interaction between N and K appeared insignificant, the results could be summarized as in Table 14: nitrogen had no influence on root infection, while for potassium a nearly significant quadratic effect was calculated (P = 0.06). In this experiment the lowest levels were not sufficiently low to cause deficiency in the plant tissues, but the highest levels were considerable overdosages.

B. N-experiments in liquid media

In order to improve standardization of root inoculation and to facilitate root observation subsequent experiments were performed in aerated liquid media. For the nitrogen experiments 2 a, b and c the equivalent ratio of the cations in the nutrient solutions was $32:48:20$ ($K^+:Ca^{++}:Mg^{++}$); the anion ratios varied according to the nitrate level applied and were for $NO_3^-:H_2PO_4^-:SO_4^{--}:Cl^-$ $5:5:35:55$, $10:5:35:50$, $20:5:35:40$, $40:5:35:20$ and $60:5:35:0$. The following quantities of trace elements were added per litre of nutrient solution (Steiner, 1966): 2,5 mg Fe as FeEDTA; 2,0 mg Mn as $MnSO_4.4$ aq; 0,02 mg Cu as $CuSO_4.5$ aq; 0,1 mg Zn as $ZnSO_4.7$ aq; 0,05 mg Mo as $Na_2MoO_4.2$ aq; 0,5 mg B as H_3BO_3.

In experiment 2a six three-week old tomato seedlings were cultivated for 27 days in each of the nutrient solutions, after which the roots of four plants of each group were soaked for 24 hours in a suspension containing 100.000 zoospores of P. nicotianae per ml, and the roots of the two controls in deionised water. After inoculation the plants were returned to fresh nutrient solution and the root systems observed several times during the following three weeks.

This experiment was repeated twice (2b and 2c) using ten plants per treatment, of which in experiment 2b the roots of eight plants were inoculated with a mycelial suspension and two plants remained uninoculated; in experiment 2c the number of inoculated plants was reduced to five, the three remaining plants being used for deter-

Table 15. The effect of nitrate nutrition on the growth and the nitrogen content of the host plant tissue and the infection of the roots by P. nicotianae.

NO$_3$ conc. (% of anion equivalent)	Root infection index			Data for expt. 2c			
					N level		
	expt. 2a	expt. 2b	expt. 2c	dry weight* of plants	in nutrient sol. (meq.)	in roots**	in stems and leaves**
5	4.8	5.0	3.0	2.28	2.5	1.62	1.46
10	3.3	4.9	2.6	3.07	4.6	2.06	1.84
20	3.8	4.1	2.4	3.21	9.7	2.48	3.10
40	4.3	4.5	3.2	2.67	18.6	3.54	3.95
60	3.8	5.0	3.8	4.98	26.2	3.48	3.46

*Dry weight per plant in grams, just before inoculation
**In % of dry matter, determined just before inoculation

Tabel 15. De invloed van nitraat-voeding op de groei en het stikstof-gehalte van het waardplanteweefsel en op de wortelaantasting door P. nicotianae.

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mination of dry matter and for chemical analysis. Inoculation was made again by soaking in a mycelial suspension.

The results of these experiments, taken two to three weeks after inoculation, are given in Table 15 and show the effect of nitrate concentration in the nutrient solution on the formation and composition of the plant tissue and a slight tendency of the root infection to be lowest at the intermediate concentrations of this ion.

As in these experiments the initial pH of 6.0 had risen to 6.6–6.8, leading to some precipitation of calcium monohydrogenphosphate, the Ca^{++} concentration in experiment 3 was reduced to 34 % of the total cation equivalents, which allowed a rise of the $H_2PO_4^-$ concentration to 8 % of the anion equivalents. Thus, the cation ratio in experiment 3 was 51:34:15, the anion ratios 6.25:8:42:43.75, 12.5:8:42:37.5, 25:8:42:25 and 50:8:42:0, respectively. The pH was standardized at 6.5 and trace elements were added as in the experiment 2. Experiment 3 was performed in duplicate each replicate containing five plants for analysis of growth and chemical composition at the time of inoculation, seven plants for inoculation and three plants as uninoculated controls. Inoculations were made on the roots, the stems and the petioles. On half of the inoculation sites of the last two parts the tissue was wounded and on the other half it was left intact.

The results of the root, stem and leaf stalk infections of this experiment are given in Table 16, while the data from the chemical analysis are shown in Table 17. Again there is a clear effect of nitrate concentration in the nutrient solution on the growth and the chemical composition of the plant tissue. Wound inoculation caused a much more rapid extension of the lesions than inoculation without wounding. There was no significant N-effect on lesion length of the petioles, though the speed of drop of wound-inoculated leaves seemed to be reduced with increasing N-level. Stem lesions extended more deeply into the pith and over a greater length in the plants containing less nitrogen. Root infection was not influenced in this experiment (Table 16).

Table 16. The influence of nitrate concentration in the nutrient solution on growth and on infection of roots, stems and leaf stalks by *P. nicotianae*.

	NO_3^- conc. in solution (% of anion eq.)			
	6.25	12.5	25	50
dry weight/plant* (g)	2.07	3.66	4.75	5.09
length of petiole lesion: (cm)				
unwounded	16.7	14.2	19.3	20.1
wounded	40.9	34.6	38.1	33.5
length of stem lesion (cm)	18.0	14.6	9.6	3.7
depth of stem lesion (% of stem diameter)	95	100	87	52
root infection index	2.1	2.1	2.4	2.4
shedding of leaves** (number of days)	6.2	11.4	12.1	14.7

*At time of inoculation
**Mean number of days between wound inoculation of petiole and leaf drop

Tabel 16. De invloed van nitraat-concentratie in de voedingsoplossing op de groei en op de aantasting van wortels, stengels en bladstelen door P. nicotianae.

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Table 17. Chemical composition of roots, stems and leaves of tomato plants grown on nutrient solutions with different nitrate concentrations (in % of dry matter).

Plant part	NO$_3^-$ conc. in solution in % of anion eq.	Total N	NO$_3$-N	P	K	Ca	Mg	total S	SO$_4$-S	Cl
roots	6.25	1.73	0.02	1.31	6.62	0.90	0.84	0.48	0.42	4.24
	12.5	1.81	0.04	1.43	6.38	1.18	0.66	0.55	0.44	3.46
	25	2.41	0.29	1.88	6.60	2.60	0.57	0.64	0.57	2.08
	50	2.98	0.94	1.84	6.47	3.06	0.43	0.64	0.48	0.07
stems	6.25	1.13	0.02	0.85	6.49	0.95	0.52	0.25	0.23	2.79
	12.5	1.22	0.02	0.69	5.86	0.85	0.39	0.21	0.18	2.17
	25	1.81	0.37	0.71	7.03	1.07	0.46	0.26	0.24	2.55
	50	3.00	1.42	0.65	8.30	0.99	0.51	0.30	0.30	0.18
leaves	6.25	2.22	0.01	0.68	3.69	2.29	0.48	0.81	0.71	1.75
	12.5	2.62	0.01	0.68	4.35	2.44	0.53	0.92	0.76	1.99
	25	3.78	0.18	0.70	5.35	2.85	0.68	1.02	0.86	1.93
	50	4.96	0.91	0.62	6.42	2.82	0.73	1.01	0.67	0.08

Tabel 17. De chemische samenstelling van wortels, stengels en bladeren van tomateplanten gekweekt op voedingsoplossingen met verschillende nitraatconcentraties (in % van de droge stof).

Comparison of the data of the chemical analysis (Table 17) shows that the nitrate content in the stems reached higher levels than in the leaves and the roots. The total nitrogen increased somewhat in the roots and the stem and increased most in the leaves. The K$^+$ content of the leaves and the Ca^{++} content of the roots became higher with higher N-levels. At the lowest N-treatment the Ca^{++} content of the leaves was relatively high and the K$^+$ content relatively low in comparison with the other parts, though the concentration of these ions in the nutrient solutions with the various N-levels was the same. Magnesium increased slowly in the leaves but decreased in the roots. Phosphorus and sulphur contents were little affected. The chlorine content in the root tissue decreased gradually with increasing N, due to the replacement of Cl$^-$ by NO$_3^-$ in the nutrient solution, but the chlorine content of the stem and the leaves remained constant except when no Cl$^-$ was added.

C. K-experiments in liquid media

The effect of potassium was studied in the experiments 4 and 5, of which the layout was identical to experiment 3. The NO$_3^-$: H$_2$PO$_4^-$: SO$_4^{--}$ equivalent ratio was 50 : 5 : 45 and the K$^+$: Ca^{++} : Mg^{++} : Na$^+$ equivalent ratios 6.5 : 34 : 15 : 44.5, 12.75 : 34 : 15 : 38.25, 25.5 : 34 : 15 : 25.5 and 51 : 34 : 15 : 0 respectively. The pH was standardized at 6.5 and trace elements were added as in experiment 2. In experiment 4 the roots and in experiment 5 the stems and the petioles were inoculated. The effect of wounding the aerial parts was also studied.

The results of these experiments, given in the Tables 18, 19 and 20, show that the K content of the roots did not get very low notwithstanding the low concentration in the nutrient solution. The dry matter production of the plants was not influenced and no

Table 18. The effect of potassium concentration in the nutrient solution on growth and K content of the tomato plant tissue and the infection of roots by *P.nicotianae*.

	K$^+$ conc. in nutrient solution (% of cation eq.)			
	6.5	12.75	25.5	51
dry weight/plant* (g)	4.27	4.31	3.77	4.49
K content of root tissue (% of dry matter)	4.54	6.51	6.51	8.96
root infection index**	3.1	3.2	3.4	2.8

*Mean of two experiments
**Three weeks after inoculation

Tabel 18. Het effect van de kalium-concentratie in de voedingsoplossing op de groei en het K-gehalte van het weefsel van de tomateplant en op de wortelaantasting door P.nicotianae.

Table 19. The effect of K-content and wounding of the plant tissue on development of lesions in stems and petioles of tomato after inoculation with *P.nicotianae*.

	K$^+$ conc. in nutrient solution (% of cation eq.)			
	6.5	12.75	25.5	51
K content in dry matter:				
leaves (% of dry matter)	4.75	5.17	5.80	6.45
stems (% of dry matter)	8.32	9.57	10.34	10.69
Mean length leaf stalk lesions:				
unwounded (cm)	2.9	2.9	3.2	3.4
wounded (cm)	5.5	5.5	5.5	5.4
Mean length stem lesion:				
unwounded (cm)	2.3	3.2	2.6	3.9
wounded (cm)	10.2	12.6	10.0	9.9
Increase of length of stem lesion after removal of cover				
unwounded (cm)	0	0	0	0.5
wounded (cm)	3.4	2.3	2.6	2.8

Tabel 19. De invloed van het K-gehalte en verwonding van planteweefsel op de lesie-ontwikkeling in stengels en bladstelen van tomaat na inoculatie met P.nicotianae.

differences in infection of roots, stems and leaf stalks were found. As in experiment 3, also in experiment 5, the lesions extended much more quickly after wounding the tissue at inoculation. Obviously the unwounded tissue of stems and petioles control one or more factors limiting extension of the lesion.

Moreover, after removal of the moist cotton wool, it could be seen that the lesions on the wound-inoculated aerial parts continued to enlarge, but those on the parts inoculated without wounding did not.

Table 20. Chemical composition of roots, stems and leaves of tomato plants grown on nutrient solutions with different potassium concentrations (means of two experiments).

Plant part	K^+ conc. in solution in % of cation eq.	% of dry matter								
		Total N	NO_3-N	P	K	Ca	Mg	Na	total S	Cl
roots	6.5	4.33	1.29	1.24	4.93	1.40	0.99	1.33	0.95	0.41
	12.75	4.48	1.34	1.14	6.81	1.40	0.80	0.98	0.83	0.35
	25.5	4.67	1.30	1.89	7.07	1.68	0.93	0.59	0.83	0.35
	51	4.29	1.36	1.14	9.02	1.13	0.79	0.10	0.90	0.38
stems	6.5	3.27	2.76	0.61	9.04	1.59	0.51	1.23	0.29	0.75
	12.75	3.38	2.89	0.66	10.28	1.50	0.50	0.95	0.27	0.69
	25.5	3.43	2.87	0.62	10.65	1.45	0.51	0.72	0.25	0.67
	51	3.85	2.63	0.61	11.38	1.48	0.46	0.09	0.31	0.64
leaves	6.5	4.88	1.59	0.80	5.13	3.79	0.73	1.04	1.44	0.45
	12.75	4.95	1.61	0.80	5.85	3.72	0.72	0.86	1.50	0.43
	25.5	5.05	1.63	0.75	6.42	3.64	0.72	0.66	1.39	0.44
	51	4.99	1.58	0.81	7.26	3.69	0.69	0.08	1.57	0.43

Tabel 20. De chemische samenstelling van wortels, stengels en bladeren van tomateplanten gekweekt op voedingsoplossingen met verschillende kalium-concentraties (gemiddelden van twee proeven).

5. Discussion

The nitrogen experiments confirmed the observations by Abdalla and Verkerk (1970) that application of nitrate to the roots increased the total N content of the plant tissue and even more the nitrate content. The leaves contained relatively more total N than the stems and the roots. The nitrate in the stem increased with the nitrogen concentration in the liquid medium from 2% to 50% of the total nitrogen in the stem. Contrary to the findings by Lepik (1940) and by Hagenguth and Griesinger (1941) with *P. infestans* on potato but in accordance with Grümmer (1955) with *P. infestans* on tomato, infection of tomato stem tissue by *P. nicotianae* decreased with higher nitrogen gifts. This pathogen does not seem to be favoured by high total N or high NO_3-N levels in the tissue. The nitrate levels in the dry matter of the plants with reduced stem lesion development were at a level which is considered normal for well growing plants by Smilde and Roorda van Eysinga (1968).

Therefore it may be concluded that in practical commerce the application of nitrogen fertilizers, desired for proper development of the crop, carries the additional advantage that stem infection by *P. nicotianae* may develop less rapidly.

The time lapse between wound inoculation of the petiole and shedding of the respective leaves is considerably longer with plants at high than at low N-levels, though the length of the leaf stalk lesions did not differ at different N-levels. Thus the formation of the abscission layer after infection seems to be influenced by the chemical composition of the tissue. This effect of nitrogen must be considered disadvantageous, as it increases the risks of stem infection.

Contrary to nitrogen, in these experiments potassium did not influence the growth of the plants. Steiner (1966) found a normal development of tomato plants at a K^+ concentration in the nutrient solution of 22% of the cation equivalents and a K^+/Ca^{++} ratio of 1/3, but in these experiments normal growth was still obtained at 6% and a K^+/Ca^{++} ratio of 1/5. Moreover, according to Roorda van Eysinga (personal communication) K deficiency in tomatoes is seldom encountered in the Netherlands. In the experiments reported above the lowest K^+ concentration was still too high.

Nevertheless low levels of K are unacceptable in commercial practice, because of reduction of the quality of the produce (Roorda van Eysinga, 1966). As no effect of K on infection by *P. nicotianae* was found, the rate of application of potassium fertilizers should be based solely on the cultural requirements of the tomato crop.

IX. Control

1. Introduction

Control of plant diseases can be attempted by measures aiming at optimal growth conditions for the host plant, at creation of an environment unfavourable for the pathogen and at reduction of inoculum density (soil tillage, host nutrition, control of temperature and relative humidity, tending of plants, weed control, pruning and tying, crop rotation, crop and soil sanitation, disinfection of equipment and soil, etc.). Furthermore, disease incidence may also be reduced by chemical and biological control and by resistance of the host plant. These aspects will be discussed successively with respect to *P. nicotianae* on tomatoes.

The ways of tilling the soil for planting a tomato crop in glasshouses in the Netherlands have never been shown to exert an influence on the infection of tomato plants. The results in chapter VIII have shown that the nutrition of the host is not very important for its infection, so that the quantity, type and method of application of fertilizers should not depend on phytosanitary requirements. Watering and soil temperature, however, are most important as shown by the results obtained in chapter VII.

The control of climatic factors inside the glasshouses, primarily temperature and relative humidity, is of paramount importance, because these factors greatly influence growth and sporulation of the pathogen. Operations like tying and deleafing of the plants and weed control should not be neglected, because they influence the micro-climate.

As shown in chapter V the pathogen is able to survive in the soil for a period of four years and more. Consequently, crop rotation on its own does not offer the prospect of adequate control on the Dutch vegetable growers' holdings, because there is only a very limited number of crops which can be grown profitably on a large scale, and because there is an increasing need for growers to specialize on crops in order to withstand competition. However, when for some reason the soil cannot be disinfected between two successive crops, rotation of crops may be desirable.

Disinfection of equipment and sanitation of the crop may reduce the inoculum concentration in the glasshouses. This may be especially important when raising young plants, as these are very susceptible. The inoculum concentration in the soil is most important and may be reduced by physical and chemical means (biological methods are as yet unknown). Protection of the plants by application of fungicides at or after planting may be equally important. Resistance of the host would be a desirable character.

In the sections 2, 3 and 4 of this chapter studies on control of infection at and after planting into the glasshouse soil will be reported. In section 5 attention will be paid to preventing infection of transplants during propagation, while preliminary data on resistance within the genus *Lycopersicum* obtained in these studies are reported in section 6.

2. Root infection

The roots of tomato plants planted in the glasshouse soil are infected by propagules residing in this soil. Control of this type of infection should therefore be directed towards reduction or elimination of this inoculum. Treatment of the soil before planting has the advantage that broad spectrum disinfectants or heat can be used. Soil treatment by these means, however, requires such an investment, that often growers who have not observed disease symptoms on the roots of the last crop tend to omit it. This system appears even more attractive to the grower when at the same time crop rotation is practiced. Thus, there is a regular demand for some method of control immediately before or some time after planting, for instance when unexpectedly the glasshouse soil or the plant material proves to be infected or when the grower begins to doubt the correctness of his decision not to disinfect his soil.

A. Soil treatment before planting

The more important aspects of the choice of soil disinfectants are the level and spectrum of activity and the required waiting period before planting. In this research project special attention has been paid to those soil sterilization methods which are used already in normal practice in the Netherlands to control root knot nematodes and corky root disease (see chapter II). Steam has been used as a general soil sterilant for years and is of course included in the experiments to be reported here.

In 1936 Godfrey reported that chloropicrin appeared to be effective against, amongst other fungi, *P. cactorum*. After 1945 this product was used increasingly in vegetable growing under glass in the Netherlands. Methyl bromide, advanced as a nematicide in 1940 by Taylor and McBeth, was increasingly used as a soil disinfectant in the Netherlands in the 1960's. Though this compound did not prove as fungicidal as chloropicrin (Stark and Lear, 1947), it aroused much interest, because it evaporates from the soil much more quickly than chloropicrin, the boiling points being 115°C and 4°C respectively. In normal practice it therefore is applied at somewhat higher rates than chloropicrin. The higher volatility of methyl bromide also allows its application at lower soil temperatures, viz. in the late autumn.

Apart from the disinfectants mentioned above, some others were tested which are used more in open fields because of their relatively low volatility (Goring, 1967). They were metam-sodium, dazomet and methylisothiocyanate.

A1. Soil treatment by heat.
The soil can be disinfected by heating above the thermal death-point of the pathogen. To determine this thermal death-point aliquots of 1200g of moist sandy soil, supplied with inoculum of the pathogen ten days before, were heated to temperatures of 45, 50, 55 and 60°C for exactly 30 mintues. The soil was subsequently planted with fourteen day old seedlings, cv 'Moneydor', in planting boxes, separated from each other by plastic sheeting on all sides. As shown in Fig. 12 the thermal death-point of the fungus is between 50 and 55°C. Replanting the soil twice with a new set of young susceptible seedlings showed, that the fungus had been permanently inactivated by the two highest temperature treatments.

In order to investigate the effect of soil disinfection by steam, agar cultures of the

Fig. 12. Infection of tomato seedlings by *P.nicotianae* from artificially infested soil, heated to different temperatures (12 plants per treatment).
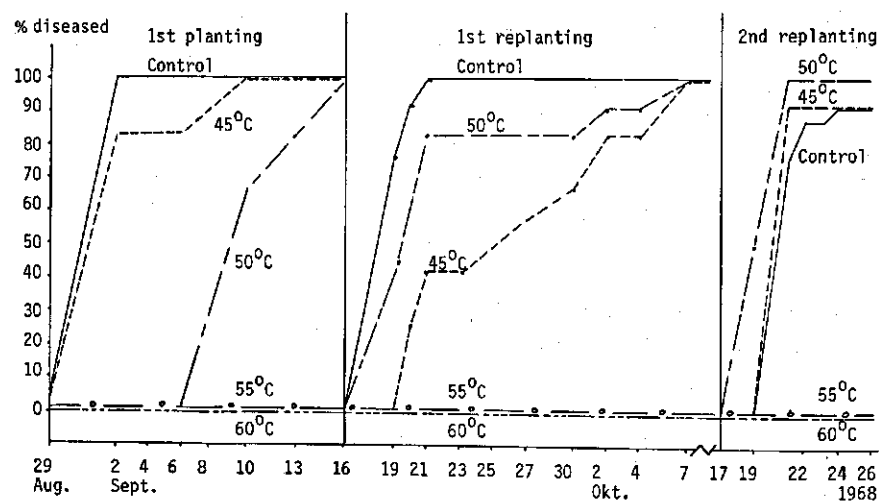

Fig. 12. Aantasting van tomatezaailingen door P. nicotianae vanuit tot verschillende temperaturen verhitte, kunstmatig besmette grond (12 planten per behandeling).

pathogen were mixed with the soil and this mixture subsequently heated in a soil sterilizer for three hours. After cooling the soil was planted with ten to fourteen day old seedlings, cv 'Moneydor'. All treatments were tested in three or more replicates per experiment. Disease assessment was carried out by counting the number of plants killed or by calculating the root infection index as described in chapter VII and the mean lifetime of the experimental plants. The latter datum will only be reported, when it adds to the information obtained from the root infection indices.

In most experiments the soil was replanted without further treatment once or sometimes twice with a new batch of young seedlings immediately after uprooting the previous batch in order to investigate the duration of the treatment effect.

The steam treatment was included in the same experiments as the treatments with soil disinfectants, which will be discussed below. The results of one of these experiments are given in Table 21. They show that control of *P.nicotianae* by means of heat is quite effective.

In some experiments the heat treatment effects were lost in subsequent plantings, an example of which is given in Table 22, experiment I. The explanation may be that during the course of the experiments the plots were contaminated from neighbouring more infested plots or that the pathogen had not been killed completely by the sterilizing agents and had become active after some time or after stimulation by the root system of the tomato plants.

To investigate these aspects steamed and unsteamed soil lots were placed in the same glasshouse compartment without spatial isolation (Table 22 expt. II). Half of these soil lots were planted with young seedlings and half left unplanted. After two months the plants were uprooted and both parts were planted anew with young seedlings. This second planting was observed for one month. The experiment was repeated, but then

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Table 21. Number of wilted tomato plants (w.p.) and root infection indices (r.i.i.) caused by *P. nicotianae* on three successive groups of 60 plants as influenced by different methods of soil disinfection.

Treatment	1st planting	2nd planting		3rd planting	
	w.p.	w.p.	r.i.i.	w.p.	r.i.i.
steam	0	1	1.3[a]	1	1.6[k]
methyl bromide	1	1	1.0[a]	12	2.9[k]
chloropicrin	2	2	1.4[a]	0	1.4[k]
metam-sodium	1	1	1.5[a]	2	2.3[k]
control	37	36	4.6[b]	59	6.8[l]

Means marked by different letters differ significantly at P<0.01.

Tabel 21. Aantal verwelkte tomateplanten (w.p.) en wortelinfektie-indices (r.i.i.) veroorzaakt door P.nicotianae bij drie opeenvolgende groepen van 60 planten onder invloed van verschillende manieren van grondontsmetting.

Table 22. The influence of a host plant crop and spatial isolation of the experimental plots on the root infection of tomato plants by *P.nicotianae* in glasshouse pot experiments (in % of diseased plants at uprooting; 60 plants per treatment)

Isolated	Expt.	Soil treatment		1st planting	2nd planting
–	I	steam		0	44
		control		98	82
–	II	steam	planted	0	66
			non planted	–	0
		control	planted	92	90
			non planted	–	65
+	III	steam	planted	0	0
			non planted	–	0
		control	planted	92	97
			non planted	–	87

Tabel 22. De invloed van een waardplantgewas en van ruimtelijke isolatie van de proefveldjes op de wortelaantasting van tomateplanten door P.nicotianae in potproeven onder glas (% zieke planten bij het oprooien; 60 planten per behandeling).

the steamed and unsteamed soil lots were kept isolated in separate glasshouse compartments, in which no other experiments were carried out.

The results of these experiments (Table 22) indicate that steam sterilized the soil properly, but that contamination of one plot by another takes place when they are not properly separated. In addition, subsequent activation of the pathogen is required before infection occurs. As a consequence, in the following experiments plots were separated by plastic sheeting and special care of hygiene was taken in order to reduce contamination.

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Table 23. Soil disinfectants applied before planting.

Common name	Chemical name	Propriet-ary name	Method of application	Concentration applied	Duration of treatment	Evaporation period
chloropicrin	trichloroni-tromethane	C.P.A.	injection	35 ml per 200 and 250 l of soil	6 days	8–18 days
methyl bromide	bromomethane	Terabol	diffusion	50, 65 and 300 g per 200 l of soil	3–4 days	10–15 days
metam-sodium (33% aqueous solution)	sodium N-methyl dithio-carbamate	Monam	injection	100 ml per 200 and 250 l of soil	6 days	8–18 days
dazomet (98% active ingredient)	tetrahydro-3,5 dimethyl-2 H–1, 3, 5-thiadiazine-2-thione		mixing	45 g per 200 l of soil	7 days	15 days
methyl isothiocyanate		Trapex	injection	100 ml per 200 l of soil	7 days	15 days

Tabel 23. Grondontsmettingsmiddelen toegepast voor het planten.

A2. Soil treatment by disinfectants. Soil lots infested with the pathogen as described under A1 were treated with disinfectants by commercial methods as given in Table 23. During the treatment the soil lots were tightly enveloped in plastic sheeting. Thereafter the chemicals were allowed to evaporate by removing the plastic and keeping the soil in a glasshouse at a temperature of 15–25 °C. These treatments were included in the same experiments as the heat treatment and the disease incidence was assessed likewise.

The results of one of these experiments, in which the first batch of test plants was uprooted three weeks and the second four weeks after planting, are shown in Table 21. They indicate that these methods of soil disinfection are about equally good as far as fungicidal action is concerned. The roots in the soil treated with metam-sodium, however, were so badly damaged that the plants hardly grew. Even in the second planting six weeks after treatment some retardation of growth was observed. In a similar experiment severe phytotoxicity was also found for dazomet and methyl isothiocyanate. The root development of the plants in soil treated with these two fungicides was very bad in the first planting, but was normal in the second, which started ten weeks after treatment of the soil.

The effect of the soil disinfection by methyl bromide was partly lost in the third planting (cf Table 21). As in this experiment plots were not isolated from each other, this observation may be explained by contamination from other plots, as described above for the steam treatments.

These experiments have shown that an application of 50 g methyl bromide or 35 ml chloropicrin per 200 l soil can give control as satisfactory as steam. These results confirm those of Upstone (1968) and of Grimm and Alexander (1971). The disinfectants, which contain or break down to the active ingredient methyl isothiocyanate, all required too long a waiting period.

B. Soil treatment at and after planting
When the soil has to be treated at and after planting, the methods mentioned in the preceding section of this chapter can no longer be used and the grower must resort to less phytotoxic fungicides. In order to disinfect the soil into which the seedling roots are first struck such fungicides would have to be applied in the planting holes before planting and around the stems after planting. This close contact with the young plant means that special attention has to be paid to the phytotoxic effect of the fungicide.

B1. Materials and methods. A large number of products has been tested sometimes in different concentrations and formulations (Table 24). During the course of this

Table 24. Formulations and concentrations of fungicides tested for soil treatment at planting.

Common name	Chemical name of active ingredient (a.i.)	Formulation and % a.i.	Proprietary name
maneb	manganese ethylene bisdithiocarbamate	80% w.p. / 10% dust	Liro-maneb / Aamagan
zineb	zinc ethylene bisdithiocarbamate	65% w.p. / 8% dust	Tritoftorol
mancozeb	complex of zinc and manganese ethylene bisdithiocarbamate containing 20% Mn and 2.5% Zn.	80% w.p.	Dithane M 45
– – –	complex of ethylene bisdithiocarbamate containing 6.4% Zn, 6.3% Mn, 4.3% Cu and 0.4% Fe.	80% w.p.	Phytam
fentin acetate + maneb	triphenyltin acetate (11.5%) + maneb (34%)	45.5% w.p.	Liro-matin
fentin acetate	triphenyltin acetate	20% w.p.	Liro-tin
fentin hydroxide	triphenyltin hydroxide	20% w.p.	Du-ter
quintozene	pentachloronitrobenzene	50% w.p.	Brassicol
fenaminosulf	p-dimethylamino benzenediazo-sodium sulfonate	70% w.p.	Dexon
chloroph-thalonil	tetrachloro isophthalonitrile	73% w.p.	Daconil
captafol	N-(1, 1, 2, 2-tetrachloroethylthio) cyclohex-4-ene-1,2-dicarboximide	80% w.p.	Ortho-Difolatan
folpet	N-(trichloromethylthio)-phthalimide	75% w.p.	Ortho-Phaltan
captan	N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide	83% w.p.	Orthocide
triarimol	α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine methanol	4% p.w.	El. 273
dimethirimol	5-n-butyl-2-dimethylamino-4-hydroxy-6-methyl pyrimidine hydrochloride	10% sol.	Milcurb
benomyl	methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate	50% w.p.	Benlate
thiophanate	1,2-bis (3-ethoxy carbonyl-2-thioureido)-benzene	50% w.p.	Orga-Topsin
thiophanate-methyl	1,2-bis (3-methoxy carbonyl-2-thioureido)-benzene	50% w.p.	Orga-Topsin-M

Tabel 24. Formuleringen en concentraties van fungiciden opgenomen in proeven betreffende behandeling van de grond bij het planten.

work new fungicides became available of which the working spectrum was insufficiently known. Therefore, some of these, mainly the new systemic fungicides were included in these experiments.

The fungicides were tested in largely the same way. Potting soil or a mixture of potting soil and a sandy glasshouse soil was mixed with a half to one comminuted agar culture ($\varnothing$ 10 cm) of the pathogen per litre. Several days later the soil was distributed into 0,6 l plastic pots and treated in one of the following ways:
1. wetting the soil in each pot with 100 ml of an aqueous suspension of a wettable powder or an aqueous solution.
2. dusting wettable powder over the soil surface followed by drenching the soil with 100 ml of water per pot.
3. mixing dust through the soil before distribution into the pots.

Immediately after treatment each pot was planted with one ten to fourteen day old tomato seedling cv 'Moneydor'.

During the experiments the numbers of plants tipped over because of root and stem base infection were counted at regular intervals. In most experiments the root infection index and the duration of the antifungal effect for each treatment were determined as described in section 2.A1.

B2. Effect of fungicides on disease development. Within the group of the dithiocarbamates the complex compounds were as effective as maneb in identical concentrations (Table 25). Zineb, however, was usually less effective than maneb in such comparisons (Tables 25 and 26); it only tended to be better when applied at 1.6 times the active dosage of maneb (Table 27).

Table 25. The influence of soil treatment with fungicides on the infection of tomato plants by *P. nicotianae* and the duration of the antifungal effect. (root infection indices for plants planted one day and seven weeks after soil treatment; 60 plants per treatment).

Active ingredient (a.i.)	mg a.i./l of soil	Way of application*	Root infection index	
			1st planting	2nd planting
fenaminosulf	167	1	1.4ᵃ	2.2ᵏ
fenaminosulf	83	1	1.8ᵃᵇ	2.0ᵏ
complex dithiocarbamate	167	1	2.3ᵃᵇᶜ	4.6ˡ
maneb	167	1	2.6ᵃᵇᶜ	4.7ˡ
mancozeb	167	1	2.8ᵇᶜ	5.2ˡ
fenaminosulf	42	1	2.9ᵇᶜ	3.0ᵏ
zineb	167	3	3.2ᶜ	6.4ᵐ
zineb	167	1	4.3ᵈ	6.5ᵐ
zineb	83	3	5.0ᵈ	7.0ᵐ
control (water)	–	1	6.7ᵉ	6.8ᵐ

Means marked with different letters differ significantly at P<0.05.
*1 = application of suspension; 3 = mixing powder through soil (see text above).

Tabel 25. De invloed van grondbehandeling met fungiciden op de aantasting van tomateplanten door P. nicotianae en de werkingsduur van de middelen (wortelinfectie-indices voor planten, die één dag resp. zeven weken na de grondbehandeling werden geplant; 60 planten per behandeling).

Table 26. Percentages of tomato plants with root and stem base infection caused by *P. nicotianae* after soil treatment with fungicides immediately before planting (45 plants per treatment).

Active ingredient (a.i.)	mg a.i./l of soil	Way of application*	% of plants infected
maneb	83	1	18ᵃ
fentin hydroxide	83	1	31ᵃ
zineb	83	1	36ᵃᵇ
maneb	83	3	56ᵇᶜ
zineb	83	3	85ᵈ
control (water)	–	1	91ᵈ
quintozene	83	3	96ᵈ
quintozene	83	1	100ᵈ

Means marked with different letters differ significantly at P<0.05
*See footnote of Table 25.

Tabel 26. Percentages tomateplanten met aantasting aan wortel en stengelbasis, veroorzaakt door P. nicotianae na grondbehandeling met fungiciden direkt vóór het planten (45 planten per behandeling).

Table 27. The influence of fungicides and their mode of application to soil on the root infection and the life time of tomato plants in weeks planted one day and seven weeks after soil treatment (60 plants per treatment).

Active ingredient (a.i.)	mg a.i./l of soil	Way of application*	Root infection index		Mean life time
			1st planting	2nd planting	1st planting
healthy control	–	1	1.0ᵃ	1.2ᵏ	6.1ᵖ
fentin hydroxide	83	1	3.3ᵇ	7.0ˡ	5.6�q
fentin acetate	83	1	3.7ᵇᶜ	6.9ˡ	5.3�q͏ʳ
zineb	133	1	4.1ᶜ	6.7ˡ	5.3�q͏ʳ
zineb	133	2	4.4ᶜᵈ	6.7ˡ	5.1�q͏ʳˢ
maneb	83	1	5.0ᵈᵉ	7.0ˡ	4.7ʳˢ
maneb	83	2	5.1ᵈᵉ	6.7ˡ	4.5ˢ
maneb	30	2	5.7ᵉ	6.8ˡ	3.9ᵗ
control (water)	–	1	6.8ᶠ	7.0ˡ	2.3ᵘ

Means marked by different letters differ significantly at P<0.05
*1 = application of suspension; 2 = dry application followed by water drench (see text on page 60).

Tabel 27. Invloed van fungiciden en hun wijze van toediening aan de grond op de wortelaantasting en de levensduur van tomateplanten, die een dag resp. zeven weken na de grondbehandeling geplant zijn (60 planten per behandeling).

Fentin hydroxide proved equally effective as maneb both at a concentration of 83 mg/l of soil in one experiment (Table 26); fentin hydroxide and fentin acetate were even significantly more so in another (Table 27, P<0.01). A combined product of maneb and fentin acetate was also more effective than maneb alone, though the significance of the difference was lower (P<0.05).

The inhibition of the pathogen by captan and related compounds (folpet and captafol) did not always differ significantly from that by maneb (167 mg/l of soil) as

Table 28. The influence of different soil fungicide treatments on the root infection index and the length of tomato plants infected by *P. nicotianae* and planted one day and seven weeks after treatment of the soil (60 plants per treatment).

Active ingredient (a.i.)	mg a.i./l of soil	Experiment a			Experiment b root infection index
		root infection index		length of plants (cm)*	
		1st planting	2nd planting		
healthy control	–	1.0^a	1.0^k	24.1^o	1.1^v
captafol	167	1.4^a	2.6^l	7.8^m	–
captafol	334	1.4^a	1.5^{kl}	7.3^s	–
folpet	167	–	–	–	4.0^x
captan	167	–	–	–	4.1^x
chlorophthalonil	167	1.7^a	2.9^l	17.0^p	2.4^w
maneb	167	2.4^b	4.8^m	12.3^{qr}	4.5^x
chlorophthalonil	334	2.6^b	1.4^{kl}	14.5^{pq}	2.7^w
chlorophthalonil	501	2.6^b	1.6^{kl}	14.2^q	2.0^w
control (water)	–	5.9^c	6.9^n	–	6.0^v

*of the first planting at uprooting.
Means marked with different letters differ significantly at least at $P<0.05$.

Tabel 28. De invloed van grondbehandeling met verschillende fungiciden op de wortelaantasting en de lengtegroei van door P. nicotianae aangetaste tomateplanten, geplant één dag resp. zeven weken na de grondbehandeling (60 planten per behandeling).

shown in Table 28. Chlorophthalonil controlled infection better or longer than maneb in two out of four experiments, of which examples are given in Table 28. In the remaining two experiments there was no significant difference between the two fungicides.

Triarimol, at the rate of 4 and 10 mg/l of soil controlled the activity of the pathogen as much as maneb (167 mg/l of soil) at the lower dosage and somewhat more ($0.05<P<0.1$) at the higher.

Fenaminosulf, which was tested in three different experiments at concentrations ranging from 21 to 167 mg/l of soil was as effective as maneb (167 mg/l of soil); the results of one of the experiments are given in Table 25.

Quintozene was ineffective, whether drenched into or mixed through the soil at 83 mg active ingredient per litre of soil (Table 26). The systemic fungicides dimethirimol, benomyl, thiophanate and thiophanate-methyl did not reduce the activity of the pathogen in the soil at all, the thiophanates not even at a dosage as high as 200 mg per litre of soil.

B3. Duration of the antifungal effect in the soil. The root infection indices of the second and third plantings show, that the effect of the dithiocarbamates has usually disappeared approximately six weeks after treatment of the soil (Table 25 and 27). Exceptions, however, did occur (Table 28). The same was true for the fentin compounds (Table 27) and for triarimol.

For captafol a higher efficacy was found in the second planting than for maneb, which also holds for chlorophthalonil in two of the experiments in which this compound was tested (cf Table 28). In the other two experiments there was no difference with maneb. For captan and folpet no such data are available.

B4. Phytotoxicity. For the selection of fungicides to be used on young plants, the growth impairing characters are of great importance. Therefore growth anomalies like discolorations and growth reductions were recorded. In several experiments the lengths of the plants were measured.

Of the fungicides equally effective as maneb fenaminosulf had to be applied at near-phytotoxic dosages and the fentin compounds actually reduced root development and growth of the test plants. Captafol, folpet and triarimol retarded growth considerably, especially after planting into recently treated soil, while captan caused considerable necrosis of the leaf margins. These characters made these fungicides unsuitable for use at and soon after planting.

Also maneb caused reduction of growth as shown by the length of the plants in Table 28, which was usually more than that caused by zineb in the same concentration. Chlorophthalonil at 167 mg/l of soil was somewhat less phytotoxic than maneb in most experiments.

B5. Application methods. The mode of application has a great influence on the efficacy of the fungicide. Not only the total quantity applied per m² is of importance, but also the distribution pattern through the soil. The fungicide is needed in the soil around the planting hole, where young plants begin to strike their roots, because in this stage the plant is the most susceptible (see this chapter, section 6). In some experiments the ways of applying the fungicides to the soil described in section 2.B1 have been compared, mainly because these require different amounts of labour. From the results in Table 25 and 26 it may be concluded that mixing fungicidal dusts through the soil (application method 3) is sometimes slightly better and sometimes worse than wetting the soil with a suspension (application method 1). The data given in Table 27 show that dusting a wettable powder on top of the soil followed by watering gives as good control as a suspension drench, provided the same amount of active ingredient is applied. The life time of the plants given in this table shows the lower efficacy of dusting a dosage of 30 mg maneb as a wettable powder, which quantity equals a dust of 2 g/m² and is often applied in normal commercial practice.

Mixing a dust formulation through the top 10–15 cm of the soil does not give a sufficiently regular distribution, especially not in lumpy soil, where the fungicide does not enter the lumps, whereas the roots of the plants do. Neither does redistribution take place. Moreover, dusting means spreading over the total surface of the soil, irrespective of the formulation of the fungicide; a drench, on the contrary, is given in and immediately around the planting hole, where it is needed first. In normal commercial practice a dose of 250 ml 0.1% maneb (80% w.p.) applied to each planting hole as a drench has been found effective. To apply the same quantity (200 mg a.i.) per planting hole by dusting a dust formulation would require 4 g active ingredient per m², or 40 g/m² of a 10% dust, taking that the drench of 250 ml moistens the soil surface in a circle with a diameter of 25 cm around the planting hole and that the required depth of penetration into the soil is equal in both methods. The use of an 80% w.p. for dusting would reduce the quantity to be dusted to approximately 5 g/m². Zineb (65% w.p.), which is used in normal practice at an 0.15 to 0.2% suspension, would likewise have to be dusted at 10–13 g/m².

As the time needed to dust quantities of 15 g/m² and more is getting prohibitive,

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dust applications are only possible using highly concentrated fungicides. For a successful infiltration wettable powders are required.

3. Stem base infection

The stem base rot described in chapter III, which was found to be caused by a combination of *P. nicotianae*, high temperatures and high soil moisture contents (chapter VII), at times levies a toll of 50 % or more of the plants within four days after planting in June or July. In view of the probability that zoospores play a vital role in this type of infection, control was directed at attempting to prevent formation, dissemination and germination of zoospores.

In chapter IV the need for free water and a low temperature shock for indirect germination of zoosporangia has been shown. These requirements are fulfilled, in practice, when growers plant during a very warm day and apply much cold water to the base of the plants immediately afterwards. Then the warm zoosporangia in the top layer of the soil are cooled down in free water. Zoospores may be liberated and enabled to swim towards the stem base leading to infection.

In conditions of normal commercial practice this process can be largely avoided, when watering is either delayed until the temperature of the topsoil has dropped to approximately 20 °C or lower, or when water is applied before planting. In both cases a sufficiently moist root ball of the transplants is required. Another way of prevention is to apply the water in small quantities, avoiding a pool of water around the stems, for instance by means of trickling irrigation.

Theoretically, planting could be delayed until the air temperature has dropped sufficiently, or very old plants could be used, which are hardened off and have become more resistant (see chapter IX, section 6). Neither method, however, is acceptable to growers because proper planning of operations does not allow delay for an unknown period, and old, hardened plants resume growth after planting with more difficulty than young plants, resulting in an undue delay of the crop.

In view of the results obtained by the application of fungicides (chapter IX, section 2.B2), reduction of germination of sporangia and/or zoospores could be expected from watering with a fungicide suspension instead of with water. Such indications have been obtained from the effect of spray residues in the soil on sporangia of *P. infestans* (Leach, 1966; Cetas and Leach, 1969). Besides, Agnihotri and Vaartaja (1967) reported the prevention of sporangial germination of *Pythium ultimum* Trow by 0.1 % aqueous solutions of $NaNO_3$, NH_4NO_3 and urea. Gooding and Lucas (1959) report a very quick encystment of zoospores of *P. nicotianae* at 32 °C and above, so that watering with warm water was expected to reduce infection as well.

Two experiments were laid out in which four week old seedlings were planted into heavily infested soil at a temperature of 25 °C and subsequently watered with 100 ml of a cold aqueous suspension of the chemical compounds noted in Table 29. Cold water was used as control. In one treatment warm water was applied. The numbers of diseased plants three and eight days after planting are given in the same table.

In this experiment infection proceeded quickly, probably because only small amounts of healthy potting soil were present around the roots of the plants, which were consequently severely damaged by transplanting. The control given by maneb was considerably better than that given by zineb especially eight days after planting.

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Table 29. Percentages of plants showing stem base and root rot symptoms after planting in soil heavily infested with *P. nicotianae* and watered with fungicide suspensions or solutions of nitrogen compounds (means of two experiments; 32 plants per treatment).

Active ingredient	Concentration or temperature	% of diseased plants	
		after 3 days	after 8 days
maneb – 80 % w.p.	0.05 %*	25	42
chlorophthalonil – 73 % w.p.	0.05 %	90	100
captan – 80 % w.p.	0.05 %	2	31
zineb – 65 % w.p.	0.05 %	26	94
water cold	±22 °C	94	100
water warm	35–39°C	39	77
$NaNO_3$	0.1 %	100	100
NH_4NO_3	0.1 %	100	100
urea	0.1 %	97	100

*% of commercial products.

Tabel 29. Percentages planten met stengelbasis- en wortelrotsymptomen na uitplanten in zwaar met P. nicotianae besmette grond en aangieten met fungicidesuspensies of oplossingen van stikstofverbindingen (gemiddelden van twee proeven; 32 planten per behandeling).

To some extent this also applied to the warm water treatment in which the percentage of diseased plants increased from 39 on the third to 77 on the eighth day. This increase in number of diseased plants, however, was due to root infection and not to stem base infection. Captan controlled the pathogen well; it was however very phytotoxic, which applied to maneb to a lesser extent. The nitrogen fertilizers had no effect whatsoever. The effect of chlorophthalonil was not very encouraging.

The results obtained in this experiment showed that hot water reduced the fungal activity temporarily. This observation supports the hypothesis that stem base rot is caused by infecting zoospores (see chapter VII), because the warm water may have prevented indirect germination of the zoosporangia or/and caused premature encystment of the zoospores. Consequently zoospores would not have been able to reach the stem base. However, they would not have been killed by the applied temperature, so that they could infect the roots as aplanospores later on. Better results of such treatments at planting may be expected when conditions after planting are somewhat more favourable to the plants and somewhat less favourable to the pathogen than in this experiment, for instance when plants, propagated in peat blocks or in pots, are transplanted into less heavily infested soil.

4. Infection of aerial parts after planting

The infection of aerial parts of the plant (leaves, petioles, stems and fruits) can be avoided by preventing a high relative humidity of the air in the glasshouses. Sporangia landing on moist wounds do not desiccate quickly and have a better opportunity to germinate and to enter the host plant than those landing elsewhere. Therefore, especially after overhead watering or when the air outdoors is very moist, the relative humi-

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dity must be maintained sufficiently low to keep plants dry. These measures are usually sufficient to prevent leaf and stem infection of mature plants under the growing conditions in the Netherlands.

Fruit infection is sometimes more difficult to control, though the same measures described above are applicable. Often air circulation near the lower trusses has to be improved by removing some leaves from the lower part of the stem. For control of wet fruit rot it may sometimes be necessary to apply a low dosage of dithiocarbamate dust, but usually the environmental control measures will suffice.

Especially for the types of infection reported in this section the use of trickle irrigation would be an excellent method of control.

5. Infection of planting material

A. Leaf and stem infection
As discussed in chapter VI, an infection of the stem of a tomato plant during propagation is nearly always correlated with infection of a leaf blade and a leaf stalk. These parts of the plant are infested, either because fungal propagules residing in the soil are transported by splashing raindrops or because leaves are actually touching the soil. The last mentioned way of transmission occurs most frequently.

Based on information about the influence of environment and the possibilities of reducing fungal activity in the soil control measures can be devised to prevent this type of infection. Firstly the propagation beds should be as free from the pathogen as possible; therefore proper sterilization is required before a new propagation season is started. Secondly, actual contact between the leaves of the young plant and the soil should be prevented. As this contact is usually brought about by too heavy a load of water on the plants after sprinkling irrigation, bending of the plants may be avoided by sprinkling for shorter periods at a time. Thirdly, transport of inoculum by means of splashing raindrops can be reduced by suspending the sprinkling lines closely above the plants and by using nozzles which produce small droplets. Fourthly, when the plants are moist, drying should be promoted by appropriate heating and ventilation. Finally, if the infection is not kept under control by the measures mentioned above a dithiocarbamate dust may be applied.

B. Root infection
In chapter VI the propagation beds were shown to be the most important source of infection. As indicated above careful sterilization is necessary. Under circumstances where the pathogen can multiply quickly, this operation may have to be repeated in springtime, that is when the beds have carried three or four complete propagation cycles.

On some holdings a regular application of a fungicide suspension or a wettable powder dust to the propagation beds has been sufficient. Dithiocarbamates or chlorophthalonil may be used for that purpose. Dust formulations are less effective as applied water does not distribute them into the top layer of the soil of the propagation beds. Furthermore, the pathogen is often most active on very moist, rather muddy sites, which often occur near somewhat raised concrete paths, which lack a separate drainage system. In those cases all rinsing water is drained unto the propagation beds and mobile machinery then tends to turn the soil into mud. Therefore, such con-

structions are to be avoided. Also, plastic sheeting has been used to keep the peat blocks separated from the soil of the propagation beds. Though this method prevents infection, the irregular watering of the pots by the unevenness of the soil under the sheeting is a considerable disadvantage.

Other minor potential sources of infection may have to be excluded, for instance by regular renewal or sterilization of the sowing soil, by hygienic storage and consumption of the potting soil, by regular cleaning of machinery and tools. Special attention will have to be paid to the boxes in which the plants are transported, because they circulate from the holding of a grower to that of a propagation specialist and vice versa, consequently with much risk of contamination. Occasional disinfection by means of a formaldehyde soak may be necessary.

Summarizing, at holdings specializing in the propagation of plant material a high level of hygiene has to be maintained to be able to garantee the delivery of disease free transplants.

6. Resistance

A. Literature
Little information is available on differences in resistance of tomato roots against either *P. nicotianae* (var. *nicotianae*) or *P. nicotianae* var. *parasitica* or any of the other pathogenic *Phytophthora* spp. mentioned in chapter III. Richardson (1941) mentioned some degree of resistance in certain wild *Lycopersicum* spp., adding however, that it was inoperative in the seedling stage. In their search for resistance Satour and Butler (1967) found a high susceptibility for 13 cultivars of tomato and 23 *Lycopersicum* and *Solanum* spp. The results were based on root inoculation experiments.

In fruits rather high levels of resistance against *P. nicotianae* were reported by Felix (1948). Later on, the same author (1953), however, tested eight *Lycopersicum* spp. and found them to be susceptible except *L. pimpinellifolium*, which was found moderately resistant. Obrero and Aragaki (1965) reported one hybrid to be rather resistant and three other commercial varieties and/or hybrids to be moderately resistant. Likewise, Barksdale (1968) mentioned large differences between susceptibility of fruits of 21 tomato varieties and breeding lines, the extremes of infection being 2% and 50%.

B. Experiments
Five to six week old seedlings of *L. esculentum* cv. 'Moneydor', *L. hirsutum*, *L. pimpinellifolium* and *L. peruvianum* were planted in artificially infested soil. Six weeks later root infection indices were calculated and found to be 10.3, 10.3, 11.7 and 9.9, respectively, which differences were not significant.

Though no resistance against root infection in the juvenile stage is known at present, the increase of the resistance of normal cultivars with age has become apparent in many experiments. To investigate this aspect further, plants of cv 'Moneydor' were raised in peat blocks for 42, 37, 32 and 28 days and planted on the same day in soil to which inoculum had been added, and which was kept at 27°C for the duration of the experiment. The numbers of plants showing stem base infection are given in Fig. 13, as well as the root infection indices at the end of the experiment. The results show a lower disease incidence, when plants are older at the moment of planting.

Fig. 13. Influence of age of tomato plants on root infection by *P.nicotianae*. Age of plants at inoculation: ●—● 28 days; ×—× 32 days; +—+ 37 days; o—o 42 days.
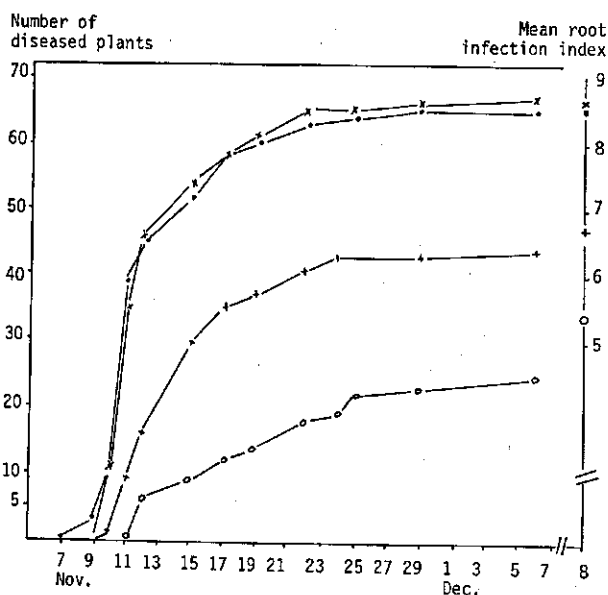

Fig. 13. Invloed van de leeftijd van tomateplanten op wortelinfectie door P.nicotianae. Leeftijd van de planten bij inoculatie: ●—● *28 dagen;* ×—× *32 dagen;* +—+ *37 dagen;* o—o *42 dagen.*

In order to observe the growth of older plants in infested soil, two month old seedlings cv. 'Extase' in peat blocks were planted in 0.6 l pots filled with a mixture of steamed sandy soil and potting soil (1 : 1). The moisture content of the soil was 38 % and was returned to that level every 2 or 3 days by weighing and watering. Half of the soil was infested artificially and the other half was kept as an uninfested control. From three weeks after planting onwards batches of 15 plants were uprooted on the dates given in Table 30 and the dry weight determined. Pots with soil but without plants were treated likewise to determine the evaporation of the soil. The water added less that evaporated was the amount taken up by the plants.

During this experiment no plants were killed by the pathogen. The data given in Table 30 show that the growth of the plants in the infested soil lagged behind by 22–23 % during the first three weeks. In the following three weeks this percentage did not change indicating a lower absolute level of growth of the infected plants and a similar relative growth in comparison with healthy plants.

The water requirement per gram dry matter produced during the observation period was lower for the infected plants than for the healthy plants. In these figures the dry root matter of the infected plants lost by the infection could not be included, so that the difference is even greater than shown in the table. These results show that in this experiment water uptake was not the limiting growth factor for the healthy plants, but it may have been so for the infected ones.

In view of data reported in the literature and collected from the present experi-

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Table 30. The influence of non-lethal root infection by *P. nicotianae* in dry matter production and water requirement of tomato plants (15 plants per treatment).

	Infected; period from planting till					Uninfected; period from planting till				
	−10/3	−17/3	−24/3	−29/3	−4/4	−10/3	−17/3	−24/3	−29/3	−4/4
Dry matter/plant at										
uprooting: in g	4.06	5.96	8.20	9.74	12.56	5.24	7.72	10.66	12.74	16.16
in % of uninfected	78	77	77	76	78	100	100	100	100	100
Water uptake/plant: in ml	640	960	1290	1395	1720	870	1365	1795	2035	2480
in % of uninfected	74	70	72	69	69	100	100	100	100	100
Water required/g of dry matter produced between 10/3 and 4/4	127 ml					147 ml				

Tabel 30. De invloed van niet-letale wortelaantasting door P. nicotianae op droge stof produktie en waterbehoefte van tomateplanten (15 planten per behandeling).

ments, the prospects of finding resistance to *P. nicotianae* in the roots of young tomato seedlings seem to be remote for the time being. The reduction of disease development with increasing age, however, is a useful asset in the control of this disease, though root infection then reduces growth.

7. Evaluation and discussion

The thermal death-point of the fungus indicates that partial sterilization at approximately 70 °C, as under investigation in several Western European countries, would be adequate for control of *P. nicotianae* in soil. As, for economic reasons this method of soil disinfection is not yet justified in normal commercial practice, steam sterilization has to be applied at 100 °C for the time being. In the Netherlands this is done by means of sheet steaming for reasons of labour economics. This method, however, has some important disadvantages. Firstly, the top layer of the soil reaches a temperature of 100 °C, but temperatures quickly decrease with depth and so does the effect of the treatment. The actual depth of proper sterilization depends largely on the capacity of the sterilization equipment and the structure and moisture content of the soil. Secondly, according to Zentmyer and Mircetich (1966), Richardson (1941) and others, the potential activity of the pathogen in sterile soil is considerably higher than in unsterile soil, due to the smaller number of antagonists in the former. As reinfestation of sterilized soil in general and of the top soil in particular remains a continuous menace, it would be better to leave the soil with as much antiphytopathogenic potential as possible (Reinmuth and Seidel, 1966) by applying, for instance, partial soil sterilization methods by which the steam air mixtures are introduced under the soil surface (cf. Last and Adams, 1966; Bollen, 1969).

Methyl bromide and chloropicrin reduced the activity of the pathogen considerably and evaporated from the soil rather quickly. Metam-sodium, dazomet and methyl isothiocyanate, however, required too long an evaporation time at soil temperatures of 15–20 °C, which is unacceptable for use in vegetable growing under glass in the Netherlands.

In the experiments on soil treatment with fungicides at and after planting, there were only slight differences of efficiency within the group of dithiocarbamates. Complex compounds were as effective as maneb, but zineb was sometimes less effective; similar results were obtained by Atkinson (1970). On the other hand, zineb was often found slightly less phytotoxic than maneb (see also Engst et al., 1968), and thus more suitable for use on holdings where crops are very susceptible to growth retardation. On soils where the plants tend to grow too much vegetatively, some growth impediment may be favourable.

Chlorophthalonil is of particular interest, because its antifungal action proved to be as good as or better than that of maneb, whilst its phytotoxic effect was at times significantly lower.

The good antifungal activity of fentin hydroxide, fentin acetate, fenaminosulf, captafol, folpet, captan and triarimol was offset by their phytotoxicity, which made them on the whole inferior to maneb for use on young tomato plants in normal commercial practice. Of these compounds fenaminosulf is particulary interesting, because at a concentration of 21 mg/l of soil it was still as effective as and not more phytotoxic than maneb at 167 mg. The concentration margin between phytotoxic and fungitoxic effect, however, was considered too narrow, as considerable toxic effects were encountered at 42 mg/l. Field tests with this compound were, according to Sonoda et al. (1970), for the same reason discontinued in California. It is remarkable, that these authors found captan ineffective and folpet as effective as mancozeb in field experiments. They report severe phytotoxicity caused by dipping 6–10 week old tomato plants in a suspension of mancozeb (5 % a.i.), whereas folpet was not phytotoxic under these circumstances.

Dimethirimol, benomyl and thiophanates did not reduce the activity of *P. nicotianae* in soil, as also described in from other reports (Delp and Klopping, 1968; Bollen and Fuchs, 1970). Also quintozene was found to be ineffective; the same is reported by Tramier and Mercier (1965) for *Phytophthora* foot rot in carnations and by Sonoda et al. (1970) for *P. capsici* and *P. nicotianae* in tomatoes.

With respect to the way of applying fungicides to the soil the general experience is that dusting a powdered formulation at a rate of 10–15 g/m² is too much time-consuming, while higher rates are prohibitive both by the material and the labour involved. The use of an 80 % maneb wettable powder may, however, be interesting, as dusting of 5 g/m² requires considerably less labour than application of a drench to planting holes individually. Dust against other diseases is often applied at the rate of 2 g/m². This dose, however, was found to give insufficient control, as shown in Table 27.

Infiltration of a wettable powder into the soil may be achieved by watering through the sprinkling lines. This does not require much labour, but may cause an unfavourable soil structure, leaching of nutrients and a decrease of the soil temperature; the last effect is a disadvantage especially in springtime.

In conclusion, under certain circumstances dusting of wettable powders followed by infiltration with water may be an economic alternative for drenching the planting holes.

The control of stem base rot, implies mainly that sudden temperature declines in the presence of free water should be avoided. Watering the soil before planting has proved to be an especially reliable method.

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Infection of the aerial parts of the host plant depends largely on the relative humidity in the micro-climate around the component parts of the plant. The gradual change-over from unheated glasshouses to heated ones, and the increasing use of automatic climate control equipment is expected to reduce the incidence of this type of infection considerably. Also planting and cultivation methods which favour unimpeded air circulation will contribute to control. Wetting the aerial parts of the host plant during watering is very unfavourable for reasons explained above, so that improvement of control should also be expected from the use of trickle irrigation methods, moistening the soil only.

A horticulturist specializing in propagation of plant material can be required to keep his product free from infection by *Phytophthora*. For this purpose soil sterilization before and during the propagation season may be needed. In the interest of the whole trade it would be of importance to initiate a classification system for plant raising specialists, comparable to that in the hotel trade. In this system hygiene at the holding should be an important criterion.

Upstone and Finney's report (1966) on resistance against *P. cryptogea* in commercial tomato rootstocks does not appear to hold for *P. nicotianae*. Some doubts about their conclusions arise, since the grafted plants may have succumbed to the disease less than the ungrafted controls because of their better growth due to the more vigourous rootstocks. This might have resulted in better regeneration of the roots, with consequences similar to those found in moist soil as discussed in chapter VII. Upstone and Finney (l.c.) did not test the resistance of the rootstock seedlings as such.

As long as resistant varieties are not available, no physiologic races of the pathogen can be distinguished. It has to be kept in mind, however, that at present two physiologic races of the black shank pathogen of tobacco, *P. nicotianae* (var. *nicotianae*), are known (Apple, 1962; Hendrix and Flowers, 1968).

In view of the lower disease incidence in older plants, control of root infection should be focussed on the first two months after planting. Consequently, a lasting reduction of the pathogen in the soil is not absolutely necessary.

X. General discussion

Before the 1960's *Phytophthora* root infection was not a problem in tomato growing under glass in the Netherlands, though the pathogen was present in the soils as shown by the incidence of wet fruit rot (see chapter III). In the early 1960's the incidence of this infection increased, reaching a maximum between 1966 and 1968. Crops in non-heated glasshouses on light soils appeared to suffer most. Holdings specializing in the production of planting material appeared to be a source of infection as described in chapter VI. In this discussion an attempt will be made to explain these phenomena, using as a basis the knowledge collected in the present studies. Though in England and on the island of Guernsey tomatoes are also grown under glass, this disease has not become a problem. This also needs an explanation.

Many of the historical data mentioned in this chapter have been collected in discussions with officers of the National Horticultural Advisory Service in the South-Holland glasshouse district and of the Glasshouse Crops Research and Experiment Station at Naaldwijk. Moreover, much information was obtained from the 'Tuin-bouwgids' published annually by the Ministry of Agriculture in The Netherlands from 1944 till 1966.

1. Incidence of root infection in the Netherlands

Before 1960 root infection by *Phytophthora* spp. probably did occur, but the plants did not suffer visibly so that the infection was not noticed.

As described briefly in chapter II planting in heated glasshouses was effected in the 1950's from the middle of January onwards with planting material grown for about three months at temperatures of 12–14°C at night and 18–20°C in the daytime. After dull days the night temperature was even kept as low as 10°C. Consequently these plants were quite old at planting, so that they had already become somewhat more resistant. Moreover, after planting the temperatures were maintained at 15°C at night and 20°C in the day. At the resulting soil temperatures the pathogen could not infect severely, and the plants were still able to grow (Table 10).

When glasshouses with better light transmissibility became available and heating systems were improved (use of oil, forced water circulation, larger number of pipes with smaller diameter), temperatures were maintained at a higher level for better use of the available light and planting was moved forward to the end of December and, later on, even to the beginning of that month. As growth in December and early January is slow (Klapwijk, 1971), light being the limiting factor, the plants remained susceptible for a longer period than at planting in January. Furthermore, growers of tomatoes in heated glasshouses began increasingly to buy their plants from specialist growers.

Another important change in the early 1960's was the replacement of 'pipe steaming' by 'sheet steaming', that is: instead of steam being introduced into the soil through dug-in pipes, it was blown under fastened plastic sheets. The first mentioned method was more laborious but usually disinfected properly a thicker layer of soil.

The less efficient soil disinfection before, the higher temperatures after planting and the subsequent higher rates of watering may have stimulated the development of *P. nicotianae* in the soil. In addition the plants themselves, because of their lower age, were more susceptible.

Growers of tomatoes in unheated glasshouses tended also to move the planting date forward, so that by 1965 planting was effected in the third week of March instead of in the first or the second week of April. At this early date soil temperatures were even more than before the limiting factor for growth. Often soil temperatures of 12–14 °C were measured, reducing vegetative growth severely (chapter VII, section 3). On sunny days, however, temperatures in these glasshouses could rise considerably for short day time periods. This could cause a temperature rise in the top few centimeters of the soil, which might activate the pathogen and the host plant. On sandy soils this top layer is often dry, so that the plants could not make use of the higher temperatures to strike new roots, especially in view of the habit of growers to water very scarcely in order to further fruit setting. Infected roots were therefore not replaced. In soil with a higher moisture content this condition did probably not occur very often.

A second reason for increased incidence of *Phytophthora* root rot in non-heated glasshouses in March and April is that by that time the soils at holdings of growers specializing in the production of planting material could have become so heavily infested that diseased planting material was produced and supplied to other commercial growers (see chapter VI). The higher temperatures were utilized to even greater extent in the propagation of transplants. This resulted in much quicker growth of the plants, increased watering, the delivery of younger, more susceptible transplants and a greater growth check at transplanting into non-heated glasshouses. Moreover, the demand of planting material for early planting into heated houses had given an earlier start to the propagation season on the specialized holdings. In addition to this, disinfection of the propagation beds before the start of the propagation season was not applied generally until the second half of the 1960's. From experiments reported in chapter VII it also appeared necessary to disinfect these beds some time during the propagation season.

The modification of the factors soil disinfection, time, soil temperature and soil moisture may have favoured the development of the pathogen in the top soil of the propagation beds, whereas the age and the check of growth of the plants at transplanting may have increased the development of the pathogen inside the host plants. In such conditions it will become increasingly necessary to propagate the transplants free of diseases and to maintain a glasshouse climate which is optimal for the plants.

2. Phytophthora root rot in England and Guernsey

In England and on the island of Guernsey the environmental conditions during the early phases of the crop were more favourable for the host plant than in the Netherlands. As described in chapter II, section 2 the transplants were often propagated by the growers themselves, at lower temperatures, usually in pots and often on raised slats; they were, and still are, transplanted to the glasshouse soil under better light conditions, allowing more rapid growth, while the air temperatures were not as high. The use of plants in whalehide pots prohibited for some time intensive contact between

Table 31. Amount of light in cal/cm² · day measured outside during the autumn and winter months in England and the Netherlands.

Month	England*		The Netherlands:**
	South Coast	Yorkshire	De Bilt
Oct.	182	135	139
Nov.	87	70	66
Dec.	58	38	42
Jan.	75	46	56
Febr.	115	95	112
March	224	196	196

*Ref. Harnett (1971).
**Ref. Koninklijk Nederlands Meteorologisch Instituut (1970).

Tabel 31. Hoeveelheid buitenlicht in cal/cm² ·dag gedurende de herfst- en wintermaanden in Engeland en Nederland.

the roots of the plants and the soil, on the propagation beds as well as after spacing out unto the glasshouse soil. An important factor was also watering, which was regular and not superfluous. This way root development was made possible, while nevertheless growth could be checked when necessary. Finally soil disinfection was applied more carefully than in the Netherlands, as dug-in pipes were used. When the crops were grown in troughs the total amount of rooting medium could be disinfected properly, as there was no connection between this medium and the subsoil under the troughs.

On the island of Guernsey *Phytophthora* root rot has been observed when plants were raised in mixtures of glasshouse and potting soil and later on when seedlings pricked out in boxes with soilless compost were used. Such so called 'boxplants' had to be transplanted early to avoid etiolation, suffered some root damage at transplanting and came into direct contact with infested border soil.

The growing conditions in Guernsey and in Southern England are very similar, though in the latter region only tomato crops were also grown in non-heated glasshouses. Then, however, planting was not done until April, when growing conditions had improved much. The data given in Table 31 show, that light conditions in winter in Southern England are better for growth of the plants than in the Netherlands, which in turn are somewhat better than those in the West Riding of Yorkshire. The great difference was that in Southern England the first tomatoes were planted in January, in Yorkshire in April, but in the Netherlands in December.

Summarizing, probably the better growing conditions for the host plants in Southern England and Guernsey and the reduced contact between the pathogen and the tomato plants were the main factors explaining the near-absence of *Phytophthora* root rot in these parts of the United Kingdom.

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Summary

Around 1960 some disorders which initially were considered to be of a physiological nature were found in tomato plants grown in glasshouses in the Netherlands. One complex of symptoms was a brown rot of the lateral roots and the tap root, often followed by decomposition of the stem base tissue and death of the plant. A second group of symptoms was the sudden death of plants within three days after planting on sunny days in June or July due to a rot of the stem base only, the root system itself staying in good order.

In 1964 and 1965 two *Phytophthora* spp. were isolated from such tissues and were identified as *P. nicotianae* v. Breda de Haan var. *nicotianae* and *P. cryptogea* Pethybr. & Laff. Later on the first mentioned pathogen was found to be also the cause of a rot of tomato stems, leaf petioles, leaf blades and fruits. This pathogen is considered identical with the *P. parasitica* Dast., known for over 50 years to be the cause of 'buckeye' rot or wet fruit rot. Fruits thus infected do not always demonstrate the 'buckeye' symptom, which therefore is considered of limited diagnostic value.

The main difference in symptoms between tomato fruits infected by *P. infestans* and *P. nicotianae* was the dry and the very wet consistency of the infected fruits respectively, for which as trivial names 'dry (*Phytophthora*) fruit rot' and 'wet (*Phytophthora*) fruit rot' are suggested. Other differences in symptoms were the absence of infection of the roots, the stem bases and the stem pith by *P. infestans*, parts which were frequently infected by *P. nicotianae*.

Whereas the first mentioned pathogen entered the glasshouses through the open ventilators infecting firstly the plants immediately beneath, the last mentioned occurred throughout the whole glasshouse as, usually, did the infected plants.

P. nicotianae and *P. cryptogea* were isolated by plating out infected plant tissue on pea dextrose agar supplemented with 30 ppm pimaricin and 10–20 ppm oxytetracyclin-HCl. The pathogen could be obtained from soil by adding a chilled 1 % glucose solution or distilled water to the soil and using green tomato fruits as bait in the supernatant. Zoosporangia of *P. nicotianae* were produced abundantly by culturing the mycelium in V8 juice/$CaCO_3$ medium and, after rinsing with water, by subsequent incubation under moist, sterile conditions. Indirect germination of zoosporangia was obtained by chilling in water to 10–12 °C. Addition of cholesterol to glucose nitrate agar or to pea dextrose agar as culture medium for *P. nicotianae* improved the shape and the content but very little the number of the zoosporangia formed.

The lesions of wound inoculated stems and leaf petioles extended much faster than those resulting from inoculations without wounding.

P. nicotianae remained virulent for at least four years in sandy border soil kept moist under glasshouse conditions. In the fourth year the inoculum potential seemed to decline.

Screening all phases of the process of propagation showed the top soil of the propagation beds to be the most important source of infection for planting material.

At the same soil moisture content root infection developed more quickly at soil temperatures between 17 and 27 °C than at temperatures below and above. Ample

soil moisture reduced the incidence of aerial symptoms, as plants were enabled to form new roots which replaced those lost by infection. With subsequent growth the plants proceeded to a more resistant stage. This effect was more pronounced at 25°C than at 14°C.

The incidence of stem base rot seemed to depend on suitable conditions for zoospore liberation in the soil. In the presence of free water these zoospores could infect vigourously. At soil temperatures of less than 20°C this infection hardly occurred.

The nitrogen and potassium nutrition of the host plants was found to have little influence on infection by the pathogen, except that ample nitrogen was found to reduce stem lesion development and to retard the abscission of infected leaf petioles.

The thermal death-point of *P.nicotianae* was found to be between 50 and 55°C. Root infection could be largely avoided, when before planting the soil was disinfected by means of steam, chloropicrin (35 ml/m^2) or methyl bromide (50 g/m^2). Such disinfection was also necessary for propagation beds in order to be able to produce disease-free planting material.

Soil treatment by means of dithiocarbamates or chlorophthalonil at or after planting reduced the activity of the pathogen sufficiently to allow the tomato plants to overcome the susceptible juvenile period. Captan, captafol, folpet, triphenyltin compounds, fenaminosulf and triarimol gave sufficient fungal control, but the level of phytotoxicity at the dosages to be applied was too great to allow their use in normal commercial practice.

When wettable powders of maneb or zineb were applied as a suspension around the stems of the plants or as a dust to the whole soil surface, the controlling effects were the same, provided equal quantities of active ingredient per planting hole were applied and the dusted fungicides were drenched into the soil with water. A quantity of 200 mg maneb (active ingredient) per planting hole thus required a dust of 5 g of an 80% commercial product per m^2; likewise a quantity of 325 mg zineb required dusting at a rate of 13 g of a 65% commercial product per m^2. A dose of 2 g of maneb per m^2, which is often applied in normal commercial practice, was not effective enough to control *Phytophthora* root rot.

Treating the propagation beds regularly with wettable powder formulations of maneb or zineb appeared a practical way of keeping this disease in planting material under control. However, a short steam treatment of these beds in February or early March would go a long way to eradicate the sources of infection during propagation.

No root resistance was found in young root stock material from *Lycopersicum hirsutum*, *L.pimpinellifolium* or *L.peruvianum*. Resistance of the tap root of *L.esculentum* increased with age and so the death-rate decreased. The same was true for *Nicotiana* spp. (*N.clevelandii*, *N.glutinosa*, *N.rustica* and *N.tabacum* 'White Burley mosaic').

The increase of the incidence of the disease in the Netherlands in the 1960's could be attributed to modification of the growing methods leading to higher levels of infestation in the glasshouse soils and to the delivery of infected transplants. The better growing conditions, the lower levels of soil infestation and the lower forcing temperatures probably brought about the lower incidence of this disease in England and Guernsey.

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Samenvatting

Omstreeks 1960 deden zich in de Nederlandse tomateteelt onder glas enkele ziekte-verschijnselen voor, die aanvankelijk aan fysiologische oorzaken werden toegeschreven. In de eerste plaats werd in 1959 (Anonymous, 1959) en in 1960 (De Boer, 1960) een ernstige aantasting van de stengelbasis van jonge tomateplanten beschreven, die optrad binnen drie dagen na het uitplanten op warme, zomerse dagen in juni en juli. Dit verschijnsel werd 'broeipoot' genoemd. In de tweede plaats meldden Verhoeff en De Mos in 1962 een aantasting van de wortels, die zich voortzette in de stengel-basis. Het eerste aantastingstype leidde soms tot een zeer hoog percentage uitval (>50%), het tweede was in het algemeen wel minder hevig, maar kon soms ernstige schade geven, omdat het tot vier à zes weken na het uitplanten in het voorjaar kon optreden, waarna inboeten niet altijd meer mogelijk was (Fig. 1).

In 1964 en 1965 werden uit aldus aangetaste weefsels twee *Phytophthora* spp. geiso-leerd, die aanvankelijk geïdentificeerd werden als *P. arecae* (Colem.) Pethybr. en een *Phytophthora* species sterk gelijkend op *P. richardiae* Buisman (Verhoeff en Weber, 1966), maar tijdens dit onderzoek identiek bleken te zijn met *P. nicotianae* v. Breda de Haan var. *nicotianae* resp. *P. cryptogea* Pethybr. & Laff.

Bij de voortzetting van dit onderzoek, dat vrijwel uitsluitend met *P. nicotianae* var. *nicotianae* werd uitgevoerd, bleek deze schimmel ook stengels, bladstelen, bladschij-ven en vruchten aan te tasten (Fig. 2 en 3). Na vergelijking van de vruchtsymptomen werd dit pathogeen identiek geacht met *P. parasitica*, een schimmel die reeds lang bekend was als de veroorzaker van 'osseogenziekte' van de vruchten. Aldus aangetaste vruchten vertoonden echter niet altijd de typische bruine, concentrische ringen, zodat deze van slechts beperkte diagnostische waarde geacht moeten worden.

Bij tuinders en voorlichters bleek verwarring te bestaan over de ziektesymptomen veroorzaakt door *P. infestans* en *P. nicotianae*. Hiertoe droeg bij het feit, dat *P. infes-tans* gedurende de laatste 20 jaar niet meer in tomaten was waargenomen en dat vruchtaantastingen zonder typische bruine, concentrische ringen ten onrechte aan *P. infestans* werden toegeschreven. De voornaamste verschillen tussen de symptomen van deze twee pathogenen zijn de consistentie van het aangetaste vruchtvlees (snel indrogend bij *P. infestans* – Fig. 4 – en lang nat blijvend bij *P. nicotianae*) en het feit dat eerstgenoemde geen wortels, stengelbases en mergweefsels aantast in tegenstelling tot laatstgenoemde (Fig. 5 en 6). Verder bleek *P. infestans* van buiten de kas binnen te komen, voornamelijk via de geopende luchtramen en de planten daar direct onder het raam het eerst aan te tasten, terwijl *P. nicotianae* verspreid in de kas voorkwam evenals de aangetaste planten.

P. nicotianae en *P. cryptogea* werden geïsoleerd uit aangetast planteweefsel door uit-leggen op erwtedextrose-agar, waaraan toegevoegd 30 dpm pimaricine en 10–20 dpm oxytetracycline-HCl. Door deze antibioticumconcentraties werd de groei van de meeste ongewenste schimmels en van bacteriën geremd, maar nauwelijks die van *Phytophthora* spp. (Tabel 2). Grote aantallen zoösporangiën van *P. nicotianae* konden worden verkregen door mycelium te kweken in V8 sap/$CaCO_3$ medium (Miller, 1955) by 25°C, dit enkele keren uit te wassen met gedestilleerd water en het dan vochtig en

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steriel te incuberen gedurende 3–4 dagen. Door een temperatuursschok met water van 10–12 °C kon er zoveel indirekte kieming van de zoösporangiën worden geïnduceerd, dat soms zoösporenconcentraties van 10^6 per ml verkregen werden. Het pathogeen werd uit de grond geïsoleerd door deze te overgieten met gekoeld water of een gekoelde glucoseoplossing (1%) en in de bovenstaande vloeistof een groene tomatevrucht te laten drijven als lokaas.

Wortelinoculaties bij vier weken oude planten van *Nicotiana clevelandii*, *N. glutinosa*, *N. rustica* en *N. tabacum* var. 'White Burley mosaic' sloegen goed aan, maar bij zes weken oude planten van dezelfde zaaisels was dit in veel mindere mate reeds het geval (Tabel 4). Bij nog oudere tabaksplanten bleek het pathogeen geen voetrot (black shank) te kunnen veroorzaken.

In vochtige, zandige grond, bewaard onder kasomstandigheden, bleek de schimmel minstens vier jaar pathogeen te blijven. Wel was de inoculumpotentiaal in het vierde jaar afgenomen (Tabel 5).

Gedurende het opkweekproces bleek de bovenste grondlaag van het kweekbed de voornaamste besmettingsbron te zijn. Direkt na een stoombehandeling van deze grond vóór de aanvang van het opkweekseizoen in oktober was de schimmel niet meer aantoonbaar, maar in de daaropvolgende maand maart was isolatie wel mogelijk. Het inoculum bouwde zich dus tijdens het kweekseizoen in de kweekbedgrond op. Dit kan een verklaring zijn voor de toename van het voorkomen van de ziekte in maart en april.

Bij een grondtemperatuur tussen 17 °C en 27 °C was de wortelaantasting heviger dan bij hogere of lagere grondtemperaturen (Tabellen 10, 11 en 12; Fig. 9 en 10). Het beschikbare vocht in de grond bleek van doorslaggevend belang voor het verloop van de aantasting. In een droge grond kon weinig hergroei van wortels plaats vinden; dientengevolge zette de aantasting zich voort tot in de hoofdwortel en de stengelbasis, waarna veel planten te gronde gingen. In een voldoende vochtige grond stelden nieuwgevormde wortels de planten in staat te groeien en een minder vatbaar stadium te bereiken (Tabellen 12 en 13).

Stengelbasisaantasting ('broeipoot') ontstond vaak, wanneer planten bij een grondtemperatuur van 20–27 °C met koel water werden aangegoten bij de stengelbasis. Door deze combinatie van vocht en temperatuursdaling werden waarschijnlijk grote aantallen zoösporen gevormd, die zich door het water naar de stengelbases konden bewegen en daar aantasten.

De stikstof- en kalivoeding van de waardplant had weinig invloed op de mate van aantasting door de schimmel, behalve dan dat een ruime stikstofgift de uitbreidingssnelheid van stengellesies verminderde t.o.v. lagere giften en de bladval na aantasting van de bladstelen vertraagde (Tabel 16). De chemische samenstelling van het planteweefsel werd aanzienlijk gewijzigd door de verschillende N- en K-doseringen (Tabellen 17 en 20).

De dodingstemperatuur van *P. nicotianae* bleek te liggen tussen 50 en 55 °C (Fig. 12). Grondontsmetting met stoom, chloorpicrine (35 ml/m²) of methylbromide (50 g/m²) bleek voldoende te zijn om in ieder geval één goede teelt te bedrijven (Tabel 22). Een goede ontsmetting van het kweekbed was van groot belang om ziektevrij plantmateriaal te kunnen kweken.

Grondbehandeling met dithiocarbamaten of chlorophthalonil tijdens of na het planten remde de activiteit van het pathogeen en veroorloofde voldoende groei aan de

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planten om een goed gewas te kunnen geven (Tabel 28). Fenaminosulf was eveneens voldoende goed werkzaam tegen de schimmel (Tabel 25), maar de concentratiemarge tussen voldoende fungistasis en te veel fytotoxiciteit werd te klein geacht om dit middel in de praktijk te gebruiken. Captan, captafol, folpet, triphenyltin-verbindingen en triarimol remden de activiteit van de schimmel bij concentraties, die fytotoxiciteit veroorzaakten (Tabel 28).

De toediening van fungiciden aan de grond als een waterige suspensie in het plantgat of bij de stengelbasis gaf even goede bestrijdingsresultaten als het stuiven over de gehele grondoppervlakte, wanneer ervoor werd gezorgd, dat in beide gevallen spuitpoeders werden gebruikt en gelijke hoeveelheden actieve stof per plantgat werden toegediend. Bovendien moest het verstoven fungicide met water ingeregend worden (Tabel 27). Bij een plantgat-dosering van 250 ml 0.1 % maneb 80 zou dan 5 g maneb 80 per m^2 moeten worden verstoven en bij een plantgat-dosering van 250 ml 0.2% zineb 65 zou dit ongeveer 13 g zineb 65 per m^2 moeten zijn. Een vaak toegediende dosering van 2 g maneb of zineb per m^2 bleek onvoldoende om *Phytophthora* voetrot te voorkomen (Tabel 27). Regelmatige behandeling van de kweekbedden met spuitpoeders van zineb of maneb bleek de schimmelontwikkeling in de grond van het kweekbed duidelijk te remmen.

In oriënterende proeven met jonge zaailingen van *Lycopersicum hirsutum, L.pimpinellifolium* en *L.peruvianum* werd geen resistentie tegen *P.nicotianae* gevonden. De resistentie van tomateplanten nam echter wel toe met leeftijd en groei (Tabel 8; Fig. 13).

De snelle toename van het optreden van *Phytophthora* wortel- en voetrot in de tweede helft van de zestiger jaren in Nederland was toe te schrijven aan een toenemende concentratie van de opkweek van plantmateriaal op gespecialiseerde bedrijven, waarbij verzieking van de bovenste grondlaag van het kweekbed in de hand werd gewerkt door het langduriger opkweekseizoen, de hogere temperatuur en vochtigheid en de onvoldoende ontsmetting van de grond van het kweekbed. Ook werd het optreden van *Phytophthora* in de hand gewerkt door de gewijzigde teeltomstandigheden na het uitplanten tot uiting komend in het gebruik van jonger plantmateriaal, hogere temuitplanten peraturen in de stookteelt en een grotere temperatuursovergang bij het planten van de koude teelt en van een minder goede grondontsmettingsmethodiek dan voorheen.

Het geringe voorkomen van de ziekte op het eiland Guernsey en in Engeland kon worden verklaard uit de betere groeiomstandigheden en de minder vroege plantdata. Bovendien was de verzorging en de hygiëne op de bedrijven daar in het algemeen beter dan in Nederland.

Acknowledgments

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