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REPLANT DISEASES OF APPLE IN THE NETHERLANDS

(Samenvatting: Herbeplantingsziekten bij appel in Nederland)

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CONTENTS

1. GENERAL

1.1. INTRODUCTION

In 1957 the Nederlandse Fruittelers Organisatie (Netherlands Fruitgrowers Organization), called the attention of the Plantenziektenkundige Dienst (Plant Protection Service), Wageningen, to the difficulties which fruit growers frequently meet when replanting apple orchards. At that time it was already known that nematodes can damage young apple trees. Therefore in 1957 Dr.Oostenbrink visited the Bangert area near Hoorn where such replant problems were common and took a series of soil and root samples. The observations made and the nematodes found did not allow a conclusion to be drawn about the cause (cf. MEIJNEKE, 1959). It became clear that the problem was complicated and that a special research programme would be necessary to solve it. The Nationale Raad voor Landbouwkundig Onderzoek, TNO, The Hague (National Council for Agricultural Research) provided funds for this work. In this way the basis was laid for a research programme with emphasis on the practical aspects of the problem. The work was started at the Plantenziektenkundige Dienst (Plant Protection Service) on 1 July 1959 and continued at the Landbouwhogeschool (Agricultural University) from 1 July 1962 onwards.

1.2. APPLE GROWING IN THE NETHERLANDS

The history of modern fruit growing in this country started with the foundation in 1898 of a national organization of fruit growers, the Nederlandse Fruittelers Organisatie. The area under apple and other fruit trees increased gradually and was extended rapidly between 1930 and 1945. Later on the area became stable and was even reduced in recent years mainly due to grubbing, not followed by replanting, of many old orchards on mixed farms. Fruit growing became more and more restricted to specialized fruit farms. The total area under pome fruit (apple, pear) and stone fruit (cherry, plum) was 47,874 ha in 1966 of which apples occupied about 33,000 ha, pears 10,000 ha, cherries 2,500 ha and plums 2,000 ha. Apple is therefore by far the most important fruit tree in the Netherlands. The average yield was about 300,000 tons of fruit a year in the period 1959 to 1965.

Table 1 gives particulars of the importance of apple growing in the main fruit centres.

Fig. 1 illustrates the situation with regard to new plantings of apple and the total apple area, in the period 1939 to 1966. The apple area has rapidly been modernized in recent years by replacement of old orchards by newly planted ones. In 1963 only 35% of the total apple area was planted with trees more than 21 years old, and 24% with trees more than 27 years old (RIJNIERSE and VAN VEEN, 1966). The oldest orchards often have standard trees. This system is no longer economic because of the varieties, the costs of tending the trees

TABLE 1. Data on the main fruit growing regions in the Netherlands (1966). Total areas planted to pome and stone fruit after VAN WELY (1967); data on the importance of apple growing provided by CBS, The Hague.

Regions	Pome and stone fruit (ha)	Planted to apple (%)	Main soil type
River clay area	19,776	70	river clay
South-Western part of the Netherlands	9,897	69	sea clay
'Bangert', near Hoorn, province of North Holland, and adjoining areas	1,549	57	sea clay, old
South Limburg	5,564	55	loess
South-East, excluding South Limburg	3,929	74	mixed, mostly light soils
North East Polder (in former Zuyderzee)	1,075	92	loam

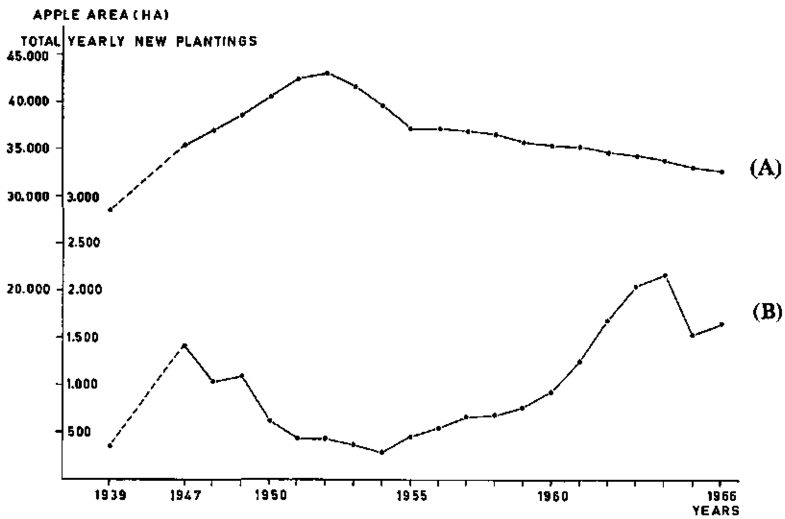


FIG. 1. Total apple area (A), and yearly new plantings (B) in the Netherlands, 1939–1966.

and the productivity. The same is true for the somewhat younger orchards of bush trees.

Most plantings of 15–20 years old and younger are composed of dwarf pyramids and similar types of trees, which are planted at smaller distances than standard and bush trees. There has also been a tendency to reduce planting distances with the dwarf pyramid system in recent years. Nowadays in most cases more than 1000 trees per ha are planted at distances between the rows of 4–4.5 m and in the rows of 1.5–3 m. Other systems are being tried, for instance the 'Groesbeek' system with 3 m between the rows and 1 m in the row. VERHEY and DE VRIES (1966) propose the growing of trees in beds with several rows of closely planted trees (for example 2 × 1.5 m) alternated with alleyways of 2.5 m.

The main reason for this interest in intensive planting systems is the need for economic production of large quantities of high quality fruit per ha, with production starting in the 2nd-3rd year after planting. The new systems not only favour high and early production, but also help to reduce costs of spraying, picking, and pruning. Most fruit farms are small: about 80% have less than 10 ha. This situation also favours the tendency towards intensive systems of fruit growing.

As regards the problems arising with replanting the following aspects of apple growing in the Netherlands are of special importance:

1. Fruit growing is now mostly restricted to specialized farms on which apple is by far the most important crop.
2. In most fruit growing regions it is difficult or impossible to find fresh land for new plantings, because suitable soils on the farm and its surroundings are already under apple.
3. The economic life span of densily planted modern apple orchards does not exceed 20 years.

Therefore it is evident that replanting is a frequent procedure in apple growing in the Netherlands and will be so in the future.

1.3. REPLANT DISEASES, INTRODUCTION AND TERMS

Problems in cultivating plants on sites where the same species has been grown before, have long been recognized (WORLIDGE, 1698). The causes of the difficulties met in maintaining monocultures vary widely. Factors such as deterioration of soil structure, erosion, chemical exhaustion, and accumulation of parasites, have led to the abandoning of important centres of single crops, such as is illustrated by the history of tobacco growing in West Virginia (CRAVEN, 1965).

Deterioration of soil structure, erosion, and chemical exhaustion, however, have largely been overcome in modern agriculture. Yet, traditional agriculture all over the world is faced with the fact that in many cases crops cannot be grown permanently as monocultures, under otherwise favourable conditions, without suffering yield losses. Other factors, parasitic and unknown, are responsible for this, and crop rotation is the basis for avoiding damage due to these replanting effects. The parasitic and unknown factors limit the possibility of simplifying rotation schemes as is often desirable because of recent developments towards greater efficiency in agriculture. In the Netherlands field experiments with annual crops clearly demonstrate the dangers involved when certain species are too frequently cultivated on the same field (HIJINK and OOSTENBRINK, 1968). An interesting case is the officially regulated crop rotation with regard to potato growing in the Netherlands, to prevent the build up of populations of *Heterodera rostochiensis* (OOSTENBRINK, 1950).

Recently resistant varieties and soil fumigation were introduced to support the effect of the crop rotation regulations in potato growing. Generally, combating unfavourable effects of the repeated cultivation of one crop by soil fumigation, is confined to intensive systems of growing horticultural crops.

The terms 'replant' and 'replanting' are especially used in connection with fruit trees and woody nursery plants, but are also applicable to other crops. These terms indicate, by definition, the second or following planting of the same or a closely related species at a given site (SAVORY, 1966). The problems involved may be caused by factors which also affect other crops (as in the case of polyphagous parasites) or may be specific, i.e. only harmful to the same, or a closely related species. In the literature 'replant problem' and 'specific replant problem' have been used, as well as other descriptive terms such as 'problems in tree replacement'. The 'specific replant problems' have been named 'specific replant diseases' by SAVORY (1966), because 'diseases' is preferable to 'problems' in view of the vagueness of the latter term. 'Specific sickness', proposed by OOSTENBRINK and HOESTRA in 1961 has the same meaning but is linguistically less satisfactory. In this publication SAVORY's term 'specific (apple) replant disease' will be used, abbreviated to 'SARD'. In accordance with this, for general indication of replant 'problems' irrespective of specificity 'replant diseases' will be used, as in the title of this publication.

In German literature the term 'Bodenmüdigkeit' is used to indicate specific replant diseases. This term is, however, only used as long as the cause is unknown. Once the cause is detected, it is preferable to adopt a new name which will indicate the causal factor. The cause of SARD is also unknown and once it is established, the position of the name might be reconsidered.

1.4. THE OCCURRENCE OF APPLE REPLANT DISEASES IN THE NETHERLANDS

1.4.1. *General observations*

It has long been known that replanted apple trees, in the first year after planting do not grow as well as trees on fresh land (VAN DER VEEN, 1918). This was not a serious economic problem in the prevailing extensive planting systems which did not normally give early yields. In modern systems of fruit growing, as described in section 1.2., high investments, and consequently early production, and high returns are required. Therefore any growth retarding factor is sharply felt, and growers have become aware that replant diseases cause damage in apple growing.

About 20 years ago very little was known about the factors involved (DE BAKKER, 1948). Positive experimental evidence first became available when the possible significance of nematodes was realized. When the relationships between nematodes and poor growth of apple were further studied, it became apparent that nematodes were not responsible for all cases of growth retardation after replanting apple. In the following section it will be demonstrated that two different replant diseases of apple in the Netherlands can be distinguished, one, not specifically harmful to apple, caused by nematodes, and the other, specific to apple, not caused by nematodes, i.e. specific apple replant disease.

1.4.2. *Factors involved*

In studies on the reproduction of, and damage done by free living root infesting nematodes OOSTENBRINK and co-workers showed from 1954 onwards, that many crops, especially woody ones were susceptible to the endo-parasitic nematode *Pratylenchus penetrans* (Cobb). It was shown that both apple seedlings and trees can be seriously damaged. The same studies indicated that the other species were not very harmful to apple. Research in other countries also point to the importance of *P. penetrans* as a nematode pest of apple. The relevant literature will be discussed in chapter 2.

It was thought possible that *P. penetrans* was largely responsible for the difficulties met in replanted apple orchards. It soon became apparent, however, that there were clear limitations to this hypothesis. In analysing results of one of his crop rotation experiments OOSTENBRINK (1956) observed that damage occurring in apple trees and roses on certain plots could not be fully explained on the basis of the nematode figures. Apple following two years cultivation of apple and rose following two years cultivation of rose showed poorer growth than expected if only the nematode figures were taken into account. So, two replant diseases not caused solely by nematodes were present on plots where the same plant species had been grown before. Other experiments were done and OOSTENBRINK and HOESTRA (1961) reported that specific replant diseases could already be demonstrated for both crops after one year of seedling cultivation.

The effect of the nematicide DD was tested in preliminary field experiments with apple in the river clay area (MEIJNEKE, 1959). The results suggested that in the orchards concerned the poor growth of replanted trees on control plots was not due to the nematodes present.

An inquiry organized by VAN MARLE (1962) gives interesting information about the situation in the river clay area. Growth of apple and pear in replant situations was evaluated. Information was collected on 164 apple orchards, planted after apple or mixed plantations of (mostly) apple and pear, and on the nematode infestation of the soils.

Although there was some effect of the presence of *P. penetrans* on growth, the results clearly confirmed that other factors too are involved. These, and other aspects of VAN MARLE's (1962) work will be discussed later in more detail. His results are similar to OOSTENBRINK's conclusions on a survey in the Bangert area in 1957 (unpublished data).

It was thus clear that in a few important fruit growing areas with clayey soils the common replant diseases can in many cases not be related to nematodes. The known good growth of other crops on apple soils in these regions, together with the observations mentioned indicate that the factor involved is the specific apple replant disease (SARD) as defined in section 1.3.

The generally high standard of cultivation in Dutch orchards and observations made, make it rather improbable that factors such as poor soil structure or deficiencies play an important role in replant diseases. More evidence will be discussed later.

1.5. Outline of the research programme

The main purpose of the research programme was to help the Dutch fruit growers to overcome the difficulties involved in replanting apple. Preliminary experiments were first carried out to get more information on the importance of replant diseases, particularly SARD, and to find indications on the methods to be followed in further research. These first experiments included studies on the effect of soil treatments. The results of these preliminary experiments were awaited before the definite research programme was established.

It became evident that SARD was a well defined and important problem. Some treatments indicated that the disease could be controlled, but the identification of the causal factor seemed to be difficult. The development of methods for practical control was given priority. It was realized that work on the control of a soil-borne disease of unknown cause can yield convincing results only if experiments are carried out on rather a large scale under a variety of conditions. So a total of 75 field experiments spread all over the important fruit growing areas was carried out. For the same reason many pot experiments were done, both for testing treatments and for studying relationships of responses in the field and in pots. The work on practical aspects was concentrated on two points, viz. 1. the study of the best chemical treatment of the soil and the conditions of application, and 2. the development of a bioassay for the prediction of SARD.

Diagnostic studies of SARD were continued throughout the research programme as a second subject. Apart form attempts to identify the causal factor directly, there have been experiments and observations directed towards further characterization of the disease to limit the field of diagnostic research.

No sharp lines were drawn between diagnostic research, and work on the practical aspects. So, many of the experiments on control gave information on the cause of SARD, the more so, because in many cases treatments were especially included for this purpose.

1.6. Materials and methods

In this section those materials and methods will be mentioned which were of general interest for the work to be described in this publication. Various details, and special techniques, will be described with the experiments concerned.

Soil samples in the field were mostly taken with a hemi-cylindrical steel borer of 2 cm diameter to a depth of 25 cm, for chemical analysis, determination of pH, and for estimating nematode populations. Samples were taken not only of control soil and soils treated with nematicides, but also from soils treated with broad spectrum fumigants and heat. The nematicidal action of the latter group of treatments was considered a valuable indication of their general effectivity.

The areas sampled varied according to experimental needs: sometimes whole fields were sampled, and, in other cases, single plots, individual trees, etc. Samples from deeper layers were taken with a shovel.

When soil was needed for pot experiments, samples of mostly 20 l and more, depending on the experiments, were taken with a shovel from a depth of 5–30 cm.

Root samples were collected with a shovel and were used for nematode extraction, isolation of other organisms, and observation of symptoms.

Chemical analysis and some of the pH determinations were carried out by the Bedrijfslaboratorium at Oosterbeek. In the other cases the pH was determined with a 'Radiometer' electrical pH meter. All pH values cited in this thesis apply to pH 'KCl', i.e. pH determined in a soil suspension in a 1 N KCl solution.

For extracting active nematodes (free living species and active stages of sedentary species) from soil, samples of 100 ml were processed in the Oostenbrink elutriator, which was used in combination with a set of five 44 μ sieves of 30 cm diameter to reduce the quantity of the suspension, and a cotton wool filter for the final separation, in a 24 hour incubation period, of nematodes from remaining soil debris (OOSTENBRINK, 1960). Active nematodes from root samples were isolated with a funnel-spray method. The extraction period was 7 days, which is satisfactory for *Pratylenchus* and other species (SEINHORST, 1956; OOSTENBRINK, 1960).

Root samples were placed in the extraction apparatus as soon after sampling as possible. Soil samples were often stored in a cold room at 5°C for some time before extraction took place; storing up to two months did not change the nematode populations.

Soil samples for pot experiments were first sieved to remove stones and roots, and then thoroughly mixed. The soil needed for each experiment, including the controls, was then put into an adequate number of 2.5 l glass jars, each with a little over 2 l of soil. There were two of these jars per treatment, for filling pots and sampling for nematode analysis, pH determinations, etc. When fumigants were applied, they were pipetted at a depth of about ten cm into the soil. For the application of other liquids and of powders the soil was taken out of the jars, mixed with the products, and put back in the jars. When necessary or desirable the jars were closed with 4-fold polyethylene sheets of 0.02 mm thickness kept in place by rubber bands. Soils treated with fumigants were kept sealed in this way for one week. For the calculation of dosages in these experiment 1 liter of soil was considered equivalent to 1/300 m^2 in the field.

The pot experiments were set up as randomized block designs with 6 or, mostly, 8 replicates. In each pot one test plant was planted. Pots used were 'long model VII' clay pots which allow for sufficient vertical growth of root systems as required by apple seedlings. Inside dimensions were: height 13 cm, diameter of top 10.5 cm and bottom 6 cm. Each pot contained 0.5 liter of soil. For the evaluation of the routine test, slightly larger pots were used. In the greenhouse plastic bags were put tightly around the pots; in outdoor experiments the pots were buried in the soil.

As test plants in most experiments 'Bittenfelder' apple seedlings of selected mother trees were used. Dried seeds were stored at room temperature or in a cold room. After stratification at 4°C (storage in moist sand) for at least 40 days the seeds germinated rapidly under greenhouse conditions. Young apple seedlings were thus available for experimental purposes throughout the year.

Seedlings were planted in the pots preferably when two leaves were formed. At this stage there is hardly any formation of side roots, and the seedlings suffer little from transplanting.

Three weeks after planting 50 ml of a nutrient solution was given to each pot. Of the major elements, N was added in a relatively high dosage (5 g NH_4NO_3/l of nutrient solution), to compensate for the nitrogen effect of soil disinfecting treatments, as will be discussed later. Per l, the following minor elements were added (mg): $CuSO_4 . 5H_2O$ 35, $Na_2B_4O_7 . 10H_2O$ 8, $MnSO_4 . H_2O$ 140, $ZnSO_4 . 7H_2O$ 35, Fe-DTPA 35. Total length of seedlings from the soil surface to the base of the final bud was measured mostly every other week. Unless otherwise stated, the data on length of seedlings in the tables apply to plants of about 10 weeks old. At the end of the experiments, 12–14 weeks after planting, fresh and dry weight of root systems and above ground parts were determined. Soil and root samples were also taken at the end of most experiments, and treated as indicated above.

In field experiments no standard design was used. On the one hand the stage of research, the nature of the treatments, and the problems to be studied, on the other hand the conditions such as available space, and preference of the owners of the fields are responsible for a variety in the size and designs of the field experiments. Roughly there are three categories. In the first, when ample space was available, several trees, mostly six, per plot were planted, with preferably six replicates of the treatments. In the second category, when little space was available, only one tree per plot was planted, but then the number of replicates was increased to ten, if possible. In these two types of experiments, fumigants were applied with the 'Shell' handinjector. In the third category, including experiments of a rather large size, tractor drawn machinery was used to apply the fumigants. For technical reasons, the plots were rather large in these experiments, and the number of replicates was limited.

In field experiments length of the newly formed shoots in each year was measured at the end of the growing season. In most cases, use was made of the Butijn branch meter, which consists of a chain and counter. This apparatus indicates the measured shoot length in dm. Stem circumference was measured in mm.

In the presentation of most tables, the results for the controls are given as average values in dm, cm, g, etc. In many cases the averages for other treatments are indicated as the ratio to the results of the control treatment in % (growth in % of controls); e.g. when growth of controls is 20, and that of another treatment 30, then the figure shown will be 150 (%). To emphasize the difference between the average values in case of the controls, and the percentages given for other treatments, results of controls are printed in italics in the columns of results concerned.

It could also be suggested to accept growth on CP treated soil as the standard treatment, to which then the growth of other treatments could be expressed in percentage of growth reduction or disease. This has been done in only a few cases. It should be realized that growth on CP treated soil is the combined result of the elimination of the causal factor, of secondary factors, and also of the nitrogen effect occurring with soil sterilization. Therefore it is not found justified to consider growth on CP treated soil as the standard to be used in expressing results. Moreover, it can not be assumed that differences in growth improvement between treatments are directly related to the degree in which the causal factor is controlled. Also in recent literature on SARD, like in this publication, growth on untreated soil is used as the basis for expressing the results of other treatments.

For statistical analysis of most experiments use was made of the Student-Newman-Keuls multiple range test (KEULS, 1952; FEDERER, 1955). The usual symbols (* and **) are used to indicate significance of differences at 95 and 99% respectively. Unless otherwise stated, the indicated significance applies to the difference between the treatment concerned, and the control.

Leaf diseases and pests were chemically controlled when necessary. In the greenhouse a preventive spraying programme (dinocap 0.08%, every other week) was applied to control mildew, *Podosphaera leucotricha* (Ell. & Ev.) Salm.

1.7. ABBREVIATIONS

The following abbreviations are used throughout this publication:

SARD – Specific apple replant disease
CP – Chloropicrin
DD – Dichloropropene-dichloropropane mixture
CBP – Chlorobromopropene
MIT – Methyl isothiocyanate
'M'Soil – Apple soil from Meteren (field experiment no. 6)

2. THE ROLE OF NEMATODES

2.1. Introduction

Part of the work on the role of nematodes has already been published (Hoestra, 1961; Hoestra and Oostenbrink, 1962). Nematodes represent one aspect of the problem of replant diseases of apple in the Netherlands, and not the most important one. They are, however, widespread and often occur together with the unknown causal factor of SARD. Therefore, a discussion of the importance of nematodes is also an essential contribution to studies on SARD.

Since the effect of nematodes on growth of apple can be evaluated, it is convenient first to discuss the role of nematodes.

2.2. Symptoms

Poor growth of the root system and above ground is a consequence of a serious attack by plant parasitic nematodes, and can be an indication of their presence. If there is a patchy distribution of poor growth over the field, nematodes are quite likely to be involved. The root system of apple is often attacked by free living nematodes (endo-parasitic and ecto-parasitic). Endo-parasites can easily be observed in the cortex of young roots (figure 2). Ecto-parasites are

Fig. 2. Young apple root infested with *Pratylenchus penetrans*, adults, larvae and eggs.

mostly not observed when root systems are examined. Their presence and the damage they may cause can only be established by other studies including an analysis of soil samples. Only in a limited number of cases do ecto-parasites cause specific symptoms, such as stubby root caused by *Longidorus elongatus* (de Man) (Kuiper, personal communication). This species, however, was only occasionally observed.

2.3. Occurrence of plant parasitic nematodes in apple orchards and nurseries

In studies on the distribution of plant parasitic nematodes in fields grown with apple, sedentary nematodes could be ruled out as factors causing poor growth of apple. The presence of nematodes belonging to *Heterodera, Meloidogyne, Rotylenchulus* and similar genera can easily be recognized because the corresponding swollen females or root galls are visible to the naked eye or under the dissecting microscope, and observations extending over several years did not reveal infestations by such nematodes. There were, on the other hand, good reasons to focus attention on free living root-infesting species. After intensive research on sedentary nematodes as causal agents of plant diseases in early plant nematology, it was realized that free living soil and root inhabiting forms can also cause serious damage. In the Netherlands, from about 1950, the free living nematodes have gained much attention and their importance, especially in the case of different species of the genus *Pratylenchus*, was soon realized (Oostenbrink, 1954).

The study of the association of nematodes with apple is complicated because, especially in orchards, grass and other cover crops are often present. This means that nematodes in the soil comprise associates of both apple and the cover crops. In nurseries apple is often grown in a clean weeding system, but rotation with other nursery crops is normal, so that here too the species found cannot be related to apple without restrictions. On the other hand it should be realized, that polyphagy is common in plant parasitic nematodes and that nematode populations built up or maintained on other crops could be noxious to apple.

Free living nematodes occur in all cultivated soils; generally a number of plant parasitic genera and species are found in any soil sample. The most common genera of free living plant nematodes in the Netherlands are: *Paratylenchus, Pratylenchus, Tylenchorhynchus, Rotylenchus* (Oostenbrink et al., 1956; Kleijburg and Oostenbrink, 1959).

Nematode populations are strongly influenced by the crop grown. Oostenbrink (1961a, 1966) and Hijink and Oostenbrink (1968) demonstrated this in more than 100 crop rotation experiments. Most crops tend to build up the population of certain species and suppress the population of others. Other factors, such as soil type, may be selective with respect to the species present and their densities. So Kleijburg and Oostenbrink (1959) in a survey of typical farms and nurseries, demonstrated that there are associations of species with crops, but also that certain nematode species are more frequent in light soils and others in heavier soils. The soil factor is important in studies of nematode damage to apple because apple is grown on different soil types. The majority of orchards is on heavier soils of different kinds (see also table 1) and nurseries are found on both light and heavy soils.

In orchards on heavier soils the generally occurring nematodes appear to be of the same genera as those in meadows and arable fields on these soils. Only

Pratylenchus penetrans (Cobb) is more frequently met in orchards than in arable land. OOSTENBRINK (1961 b) quotes figures from a survey by the Bedrijfslaboratorium at Oosterbeek which showed that in 100 ml soil samples from 271 fruit orchards in 74% of the cases the nematode was found. The frequency with which it occurred was about the same in clay and lighter soils. But the level of infestation was usually higher in light soils than in heavy soils. From 292 samples from orchards in the river clay area 60% were infested (VAN MARLE, 1962). In contrast to these figures, the Bedrijfslaboratorium showed that of fields of arable crops on clay soils only 20% were infested. In later work it became evident that in many orchards on heavier soils the observed populations of *P. penetrans* were very often a mixture of *P. penetrans* and the recently described *P. fallax* Seinhorst (SEINHORST, 1968). *P. fallax* closely resembles *P. penetrans* from which it can only be distinguished by the annulation around the tail tip. The occurrence of this new species has also been reported from England (PITCHER et al., 1966).

For other species and genera VAN MARLE (1962) gives details of 226 samples of young orchards none of which was heavily infested with *P. penetrans* (less than 55 nematodes per 100 ml of soil). From these figures the distribution frequency of these species was calculated (table 2).

TABLE 2. Occurrence of some species and genera of nematodes in orchards in the river clay area, calculated from data by VAN MARLE (1962).

Nematode	Percentage of orchards infested per soil sample of 100 ml
Pratylenchus crenatus	28
P. neglectus	26
P. thornei	57
Paratylenchus	85
Tylenchorhynchus	76
Rotylenchus/Helicotylenchus	72

Pratylenchus thornei Sher and Allen, and *P. neglectus* (Rensch) are typical species of heavier soils (KLEIJBURG and OOSTENBRINK, 1959), reproducing on different arable crops. *Pratylenchus crenatus* Loof occurs in all types of soil.

Most orchards surveyed by VAN MARLE (1962) had grass as a cover crop and moreover all these young orchards in the river clay area were planted on former orchard soil that had also been covered with grass for many years. The fact that the species and genera found are common on clay soils, especially meadows, suggests that a specific association with apple is not the case. In the Bangert area near Hoorn the clean weeding system in orchards is common, in old plantings as well as in new ones. The nematode populations of all four common genera are low in these orchards. This is especially true of *Paratylenchus* spp., which are only numerous under grass.

In 1962 trials were made to test the suitability of pot experiments to predict the occurrence of specific apple replant disease (SARD) in the field (see section

4.6.). Apple seedlings were grown from June to September. The nematode populations in the untreated pots were checked before and after growing apple seedlings on 46 apple orchard soils and 12 fresh soils, in March and in September 1962.

The average nematode populations did not differ markedly between the two groups of soils. During six months cultivation of apple seedlings (weeds were regularly removed from the pots) the level of infestation decreased for most species and genera. This was especially the case with *Paratylenchus* spp. which were reduced to about 20% of the original population in both apple and fresh soils.

Less frequently other species were found in orchards, viz. *Hemicycliophora, Criconemoides, Longidorus, Trichodorus* and *Xiphinema* species. These ectoparasites were quite rare, and populations present were mostly low. Therefore, the possibility that these genera are an important cause of replant diseases of apple can be excluded. Populations of *Longidorus* and *Xiphinema* are not quantitatively isolated from soil by the standard method mentioned in section 1.6. Therefore a number of samples have been treated with a method similar to the one described by D'HERDE and VAN DEN BRANDE (1964). As already said, *Xiphinema* and *Longidorus* spp. were not frequently found, and these species also seemed to be associated with grass rather than with apple.

Summarizing it can be said that in apple orchards nematode populations occur which differ little from the ones found in other fields of the same soil type, notably meadows. On heavier soils *P. penetrans* is more frequent in orchards than in other fields, which may be explained by its transportation with the roots of young trees from infested nurseries on light soils (OOSTENBRINK, 1957).

2.4. IMPORTANCE OF NEMATODES

Information about the possible damage done by the generally occurring nematodes in orchards on river clay soils can be derived from the above quoted survey by VAN MARLE (1962). The author examines the correlation between the nematode figures and the reports on the growth of the replanted trees. Only one species, *Pratylenchus penetrans*, showed some relation to growth of the trees. In 25% of the orchards a heavy infestation (more than 50 nematodes per 100 ml of soil) was detected, and of these 73% showed poor growth. With the non-infested orchards (40%) there were fewer cases of poor growth on an average, namely 50%. The cases with less than 55 *P.penetrans* were examined separately for the other species and genera. *Pratylenchus crenatus, P. thornei* and *Rotylenchus* spp. were more frequent in good orchards than in poor ones and numbers in good orchards were sometimes high. *P. neglectus* was rare; high numbers were found in some good orchards. *Paratylenchus* and *Tylenchorhynchus* species were frequently present in most orchards irrespective of the condition of the orchards; this suggests that growth is not much affected by these genera though information on the species is lacking.

Further information about the role of nematodes is derived from the effect of the treatment of soils with specific nematicides on growth of apple. For this purpose in most cases DD (dichloropropane-dichloropropene mixture) was used; adequate dosages are known to kill nematodes without markedly affecting many soil fungi and bacteria. From the frequent application of DD in pot and field experiments it could be definitely concluded that growth of apple was not much improved by killing the nematodes on clay soils with or without SARD.

A compound which would kill other soil organisms without affecting the nematodes, i.e., a compound with an effect complementary to DD, would be extremely useful in further assessing the role of nematodes separately from specific apple replant disease. Such a compound has not yet been found or developed. Therefore experiments on clay soils not planted with apple (fresh soils), but infested with nematodes, are useful to give an insight into the role of nematodes. Table 3 gives information on 9 different pot experiments with fresh soils treated with DD. The soils are infested with representatives of the above four main genera of plant parasites. Nevertheless the growth of control plants is good and the effect of killing the nematodes by DD is limited. These experiments confirm that on clay soils apple is not very susceptible to common nematodes present in these soils.

TABLE 3. Nine pot experiments with fresh soils. Nematode infestations per 100 ml of soil at the beginning of the experiments and effect of DD treatment on the growth of apple.

Total *Pratylenchus*	135	80	205	195	10	130	90	480	175
P. penetrans				70	5	35			40
P. crenatus		40		125		50			135
P. thornei	135	30	190			45	90	350	
P. neglectus		10	15		5			130	
Paratylenchus	115	90	465	725	30	20	160	325	435
Tylenchorhynchus	30	20	55	180	5	5	40	120	695
Rotylenchus/Helicotylenchus	10	70	95	385	20	25	20	100	305
Other *Tylenchidae*	190	120	495	460	350	175	215	300	420
Saprozoic spp.	555	385	1225	1425	1445	1060	455	585	2265
Growth of plants in DD treated soil in % of controls	98	102	124	113	111	107	139	125	106

It is therefore clear that no correlation between the occurrence of plant parasitic nematodes and poor growth of replanted orchards could be established on heavy soils, with the exception of some influence of high numbers of *Pratylenchus penetrans.*

So far attention has been paid to nematodes on clay soils, on which even *P. penetrans* seems to cause little damage. In the case of light soils it is known that *P. penetrans* can cause serious damage to apple and other plants. It is of interest here to mention how information on the significance of this species has been gained in the Netherlands in the course of years. In 1954 OOSTENBRINK demonstrated the importance of free living nematodes in Dutch agriculture and horticulture. In his first article *P. penetrans* was quoted with regard to damage in potato. It became apparent (OOSTENBRINK, 1955) that several woody nursery crops, such as *Rosa canina, Prunus* spp. and apple seedlings, are highly susceptible to *P. penetrans.* Growth of apple seedlings was strongly reduced by inoculation with this nematode. Several other crops, including wheat, oats and rye, were found to be insusceptible to damage but to be efficient host plants. This means that *P. penetrans* thrives in the roots of these plants without causing much damage. These crops therefore tend to build up dense populations. Further studies on the same experimental fields on light soils (OOSTENBRINK, 1956) showed that apple trees, rose seedlings and potato were damaged by *P. penetrans*; there was a clear correlation between the nematode numbers found in the soil at the beginning of the season and the extent of the damage.

As the nematode is endo-parasitic infestation may be spread with planting material of woody crops (OOSTENBRINK, 1957); damage was, however, not observed with first plantings of such infested material on uninfested land. More field experiments, all on light soils, were established to study the population dynamics and importance of *P. penetrans.* A total of 164 different plant species and varieties were grown on plots treated with nematicides, and on control plots (OOSTENBRINK et al., 1957). The experiments showed that *P. penetrans* is very polyphagous; no plants were found to be completely free of infestation of the roots. It was confirmed that the efficiency of the host plant is often not proportional to its susceptibility to damage. Several species of plants, such as the cereals already mentioned and also many leguminous crops, contained high numbers of *P. penetrans* in the roots but showed little damage. Much attention was paid to woody nursery crops; 84 different species and varieties were tested. Of these, only 23 were not definitely shown to be susceptible. After the harvest of the crop, at the end of the growing season, the nursery crops usually do not leave high infestation levels in the soil. This is probably mainly due to the rather small root mass of these crops as compared with arable crops such as cereals, which build up high populations. The number of *P. penetrans* per unit weight of roots of nursery crops may be as high as in cereals. Apple seedlings and eight different Malling rootstocks were among the crops tested. On one of the rootstocks no conclusion could be drawn. All the others were susceptible (M. VII) or hightly susceptible (M. I, II, IV, IX, XI, XVI, and seedlings). The number of nematodes per 10 g of roots was more than 1,000 in all these cases.

In one of a series of field experiments on light soils discussed by OOSTENBRINK, (1961b) apple seedlings were used as the test crop on plots with different levels of infestation. Significant regressions were found between nematode numbers and the growth (evaluation) of the crop. HOESTRA and OOSTENBRINK (1962) demonstrated a strong regression of growth of apple trees on the preplanting nematode density for two experimental fields on light soils and with four scion/rootstock combinations (figure 3). In the case of an orchard in the southern part of the country, also on light soil, the authors found growth reduction where it was infested with *P. penetrans.* Severity of damage was related to the

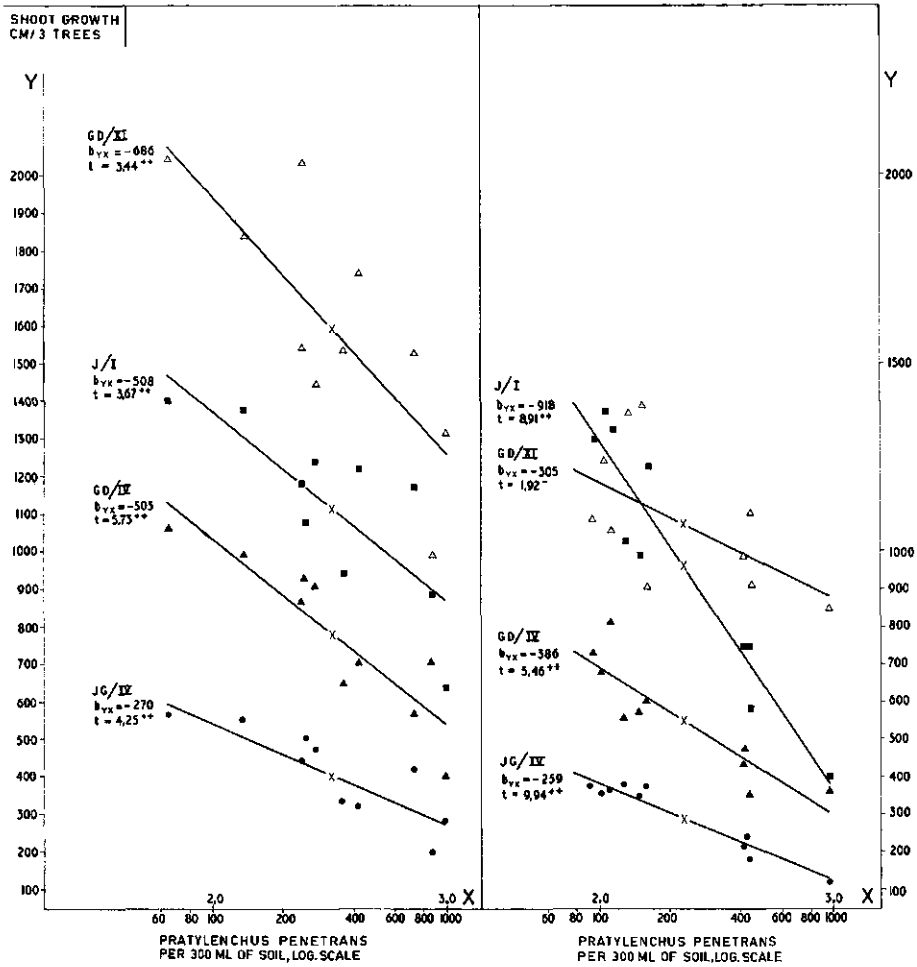


FIG. 3. Relation between the infestation level of *Pratylenchus penetrans* in the soil at the start of two field experiments, and growth of apple trees on Malling rootstocks I, IV and XI. GD: Golden Delicious, J: Jonathan, JG: James Grieve. (After HOESTRA and OOSTENBRINK, 1962)

numbers found in the roots. The population of *P. penetrans* had been built up by different arable crops, viz. rye, clover, and peas. In the North East, a field experiment was established on a light soil infested with *P. penetrans*; the results of this experiment (no. 25) are described in section 4.3.7.

Investigations in other countries yielded similar results. In Belgium D'HERDE and VAN DEN BRANDE (1962, 1963) and D'HERDE and COOLEN (1966) reported cases of severe damage to apple in nurseries and orchards by *P. penetrans*. High numbers of *P. penetrans* were frequently found on light soils in the roots of poor growing trees at several occasions, and fumigation with the nematicide DD was followed by healthy growth of the trees. Thus the authors consider *P. penetrans* to be the main factor in replant problems on light soils, but, the existence of SARD has also been observed in Belgium (GILLES, 1968).

DECKER (1960a) investigated replant diseases in three different nurseries on light soils in the German Democratic Republic. *P. penetrans* was present in high numbers, with a maximum up to 1,500 per 100 ml of soil at a depth of 16–24 cm. When inoculation of *P. penetrans* in pot experiments resulted in the establishment of the nematode, the growth of apple was seriously affected. In another publication DECKER (1960b) states that *P. penetrans* has a preference for light soils, and is particularly harmful to many woody nursery crops. The build up of the nematode population by these latter crops is relatively slow, due to the scanty root system.

In replant diseases of apple in New York State *P. penetrans* is considered to play a major role. The nematode is widely spread in orchards, which are mainly on light soils (MAI et al., 1957; BRAUN et al., 1966). Apple seedlings were severely damaged by the nematode in inoculation experiments at different temperatures (MAI, 1960). Treatments with the nematicides ethylene dibromide, DD, and dibromochloropropane resulted in improved growth. These products are recommended to combat replant diseases in orchards (PARKER et al., 1966).

Thus, the pathogenicity of *P. penetrans* towards apple is recognized by authors in different countries. Poor growth of apple was associated with the presence of the nematode and correlations were found between nematode numbers and damage. Inoculation trials led to reproduction of symptoms. Treatments of the soil with nematicides resulted in much improved growth.

The inoculation trials quoted so far were not done under sterile conditions. PITCHER et al. (1960) were not able to induce poor growth of apple seedlings by inoculation of *P. penetrans* under sterile conditions. On the other hand, various authors compared the effect of the added nematode suspensions with nematode free supernatants of the same suspensions, and showed that no growth reduction occurred if the nematodes were not present. DECKER (1960a) stresses the point that no poor growth occurred when *P. penetrans* did not establish itself after addition of the suspensions. This suggests that other organisms added with the suspension were not able to produce the symptoms in the absence of *P. penetrans*. In our own laboratories, experiments by LEBBINK (personal communication) suggested that under conditions of at least initial sterility inoculation of *P. penetrans* resulted in growth reduction of apple seedlings.

2.5. DISCUSSION AND CONCLUSIONS

The most important nematode in apple orchards is *Pratylenchus penetrans*. Its ability to cause serious damage to apple grown on light soils is evident. On heavier soils, the nematode is rather widespread but does not seem able to cause much damage. The numbers in the soil vary, especially with light soils, and have been reported to be low, even in cases where damage to trees was severe (HOESTRA and OOSTENBRINK, 1962). With this endo-parasitic nematode, numbers in the roots are a better indication of the damage it causes. In root samples from light soils, often between 5,000 and 10,000 nematodes per 10 g of roots are found. Samples from orchards on heavy soils never revealed an infestation rate of more than about 1,000. This effect of soil type may well be related to the nematodes' mobility which is very likely restricted in heavy soils, thus making it difficult for the nematode to reach the roots, so that these more or less escape damage. The prevalence of other nematodes in light soils has also been reported (REBOIS and CAIRNS, 1968; SHER and BELL, 1965).

The importance of soil type with regard to damage done by *P. penetrans* has also been stressed in other countries, as was shown in section 2.4. An interesting example is peach replant disease in Ontario, Canada. Here *P. penetrans* plays the most important role. Striking differences were observed in severity of the problem between the two main peach growing areas, Niagara Peninsula and Essex County. MOUNTAIN and BOYCE (1958) studied the backgrounds of these differences. *P. penetrans* was common in both areas, but high populations were much more frequent in Essex County, where the problem was also much more important than in Niagara Peninsula. The only systematic difference found between the two areas was the soil texture. In Essex County the orchards are generally located on lighter soils than in the Niagara area. A survey of all commercial peach nurseries in Ontario also showed a clear relation between soil texture and *P. penetrans* densities, coarse soils being more heavily infested than silt and clay soils. An interesting inoculation experiment showed that *P. penetrans* caused little damage and did not multiply well in heavy soil, whereas under otherwise comparable conditions on light soils a high rate of multiplication occurred. The important conclusion from this work is, that *P. penetrans* may be widely spread on heavier soils without causing much trouble, and this is in agreement with the observations made in the Netherlands.

The absence of an appreciable effect of DD treatment of heavier soils on growth of apple shows that nematodes are not an essential factor in the disease etiology in the cases concerned. But a further conclusion of DD effect with regard to the role of nematodes should be drawn with care. In many cases SARD is also present. This disease may mask the effect of the nematodes and of the nematode killing. Generally, if two different organisms or groups of organisms act together to cause poor growth of plants, independently from one another, controlling one of the two will have little effect if the other by itself is already capable to cause a markedly reduced growth. But, in our case, it has been shown that when nematodes are alone present, for instance in fresh soils, the influence of the nematodes on the crop is small. Therefore, also in view of the great number of cases in which the limited effect of DD on growth was demonstrated, we conclude that on heavy soils the role of the nematodes as a cause of replant diseases is small as compared to the causal factor of SARD.

In conclusion it can be said that apple orchards are infested with a range of species and genera of free living plant parasitic nematodes. The populations found were mostly not very different from those in other fields, not planted to apple, but of the same soil type. Many of the nematodes found depend on the cover crop present (mostly grass) rather than on apple. In clean weeded orchards on heavier soils the populations were low. *Pratylenchus penetrans* was more frequently found in orchards on heavier soils than on arable fields of the same soil type. The nematode is probably frequently transported with plant material from nurseries in light soils to these orchards. Evidence was presented that this species is not causing much harm to apple trees on heavier soils, and is not involved in a complex relationship with the factors causing SARD. On light soils the pathogenicity of the nematode was confirmed.

In some cases other nematodes may be of importance, such as *Longidorus elongatus*, and *Hemicycliophora* spp., but these are not commonly present in high numbers.

3. SPECIFIC APPLE REPLANT DISEASE, GENERAL ASPECTS

3.1. Symptoms and characteristics

The main symptom of SARD is a non lethal growth reduction of above ground and underground parts of both seedlings and trees. This indicates that the symptoms are not distinctive; SARD is mainly characterized by the conditions in which the disease occurs. Therefore, in this section, symptoms and other characteristics are discussed together.

The retardation of growth on apple soil as compared to fresh soil often is considerable; growth reduction of up to 90% has been observed. In the following sections and chapters, with the discussion of pot- and field experiments, numerous examples of this quantitative aspect will be mentioned. The top/root ratio of replanted apples seedlings has been reported to be reduced (Savory, 1967) but in our own studies with seedlings this has not found to be a constant phenomenon (cf. tables 9, 10 and 11). In severe cases trees planted in the field show the first symptoms as soon as the first leaves are developed. Leaves are small and cupped. In less serious cases growth is first normal. In either case growth stops early in summer, and therefore the differences with trees which are not affected and which thus continue to grow or have a second flush of young shoots in late summer, become more and more evident towards the end of the growing season. The symptoms usually occur rather uniformely distributed over the whole field, while in the case of nematode attack a patchy distribution is more common. If in cases of SARD differences in disease intensity are observed, these often apply to whole rows, and can then be connected with wide interspaces between the rows of the former planting, former alleyways, etc. Sometimes the effect of the individual sites of former trees was observed. (cf. Holland and Greenham, 1966). Poor growth on apple soil was only occasionally accompanied by well defined deficiency symptoms on the leaves. In a few cases magnesium deficiency was observed. The normal dark colour of the leaves of replanted trees indicates that nitrogen deficiency is not related to SARD. Analysis of leaves and stems of apple seedlings in some of our pot experiments did not reveal any differences between plants grown on apple soil and on succesfully treated soil with regard to Zn, Bo, Mn and Cu.

Stimulation of the formation of shoots at the lower part of the stem of trees as reported by Fastabend (1955) was not observed with the smaller dwarf pyramids in our experiments.

In the second year after planting the trees show a marked recovery; the leave symptoms disappear and new shoots are normal. The size of the tree remains reduced; often the trees in the second year are of about the same size as trees on fresh or succesfully treated soil in the first year.

Observations on seedlings were mainly made in pot experiments. The seedlings are planted in a young stage, preferably when the first two leaves are present and development of lateral roots is scarce. Growth is normal during the first 2–4 weeks after planting. Death of seedlings was rare, and not more frequent on diseased than on fresh or healthy soil. This damping-off was therefore concluded not to be related to SARD. After 2–4 weeks the symptoms become apparent, especially when a comparison with healthy plants can be made. Growth is retarded and new leaves are becoming smaller. Shortening of the internodes is more pronounced than in the field. Often very small leaves are formed over a considerable period of time. Typical rosette formation around the final bud thus often occurs on seedlings of two to three months old.

Root studies were made on seedlings. A few days after planting lesions can be observed. The root system continues to develop and after two weeks the roots become more clearly affected. A complete check to growth does not occur. Even a plant that is seriously diseased, will form new rootlets from time to time. Root hairs are reduced in size and number especially where the rootlets are in close contact with soil particles. Here also discolouration starts. The rotting process is usually confined to the primary cortex and epidermis. These often rot away and are sloughed off soon, leaving an apparantly unaffected, thin and light coloured stele, covered by remnants of the cortical layer just outside the endodermis. This layer has cells of a special structure, with thick radial walls, and occurs frequently with rosaceous plants (réseau de soutien sus-endodermique, VAN TIEGHEM, 1891). The roots may survive, and after formation of secondary cortical layers, have a normal healthy appearance. In a later stage, many of the new rootlets formed either rot away completely when 5–20 mm long, or do not grow more than a few mm long and then become distinctly swollen. Some aspects of symptomatology are shown in figure 4. Roots in disinfected soil are completely different. There are many light coloured feeder roots, with numerous root hairs, and no discolourations. The primary cortex remains intact until secondary layers are formed.

In root observation boxes filled with apple soil on top of sterilized soil, it has frequently been observed, that roots at the stage in which epidermis and cortex have ceased to function, when reaching uninfected soil, develop there into healthy parts of the root system with side roots bearing many root hairs. This shows that the remaining stele enables food transport to the root tips. And also, good growth of the above ground parts of the plants concerned, indicates that transport of food and water in the opposite direction is likewise not seriously disturbed in the affected part of the root system in apple soil.

A difficulty in comparing root systems in fresh and apple soil is that in untreated fresh soil similar root discolourations and rots occur (cf. ROGERS and HEAD, 1966), be it in a less serious degree. This may be related to the activity of mildly parasitic organisms and the normal short functional period of feeder roots (60 days) as described by BOSSE (1960).

Microscopical examination of sections of young roots of plants affected by SARD reveals that browning of the cortical tissues start with the epidermis and the endodermis. Both tissues can turn brown completely before the other cortical cells are affected. This phenomenon has also been observed in the case

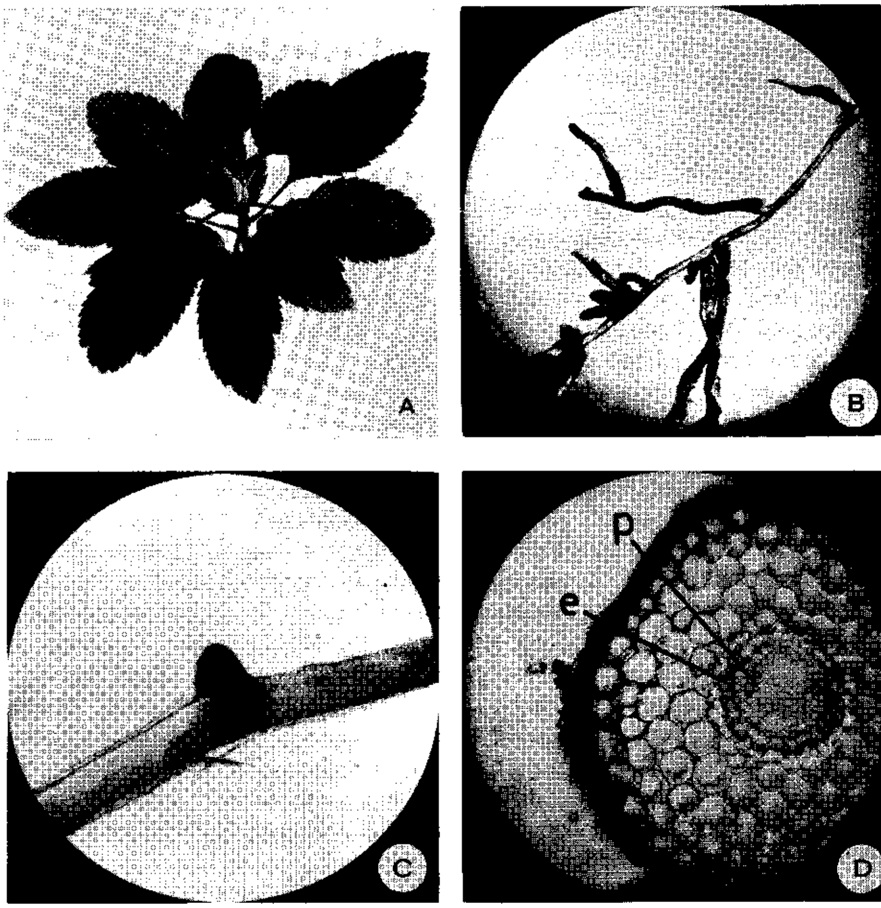


FIG. 4. Symptoms of specific apple replant disease. A: Seedling showing short internodes and small terminal leaves. B: Part of a root system. Central 'main' root of which cortex has disappeared. Lateral roots with necrotic epidermis and cortex. C: Detail of root with remainders of 'réseau de soutien sus- endodermique' and necrotic, very small feeder root. D: Cross section through young root showing necrotic epidermis and necrosis of endodermis (e) starting close to primary vascular strand (p).

of infection by *Pratylenchus penetrans* and was attributed by PITCHER et al., (1960) to the breakdown of the polyphenols present in the epidermis and endodermis but not in the other cortical tissues. In the endodermis the cells nearest to the primary vascular strands turn brown first (fig. 4). In the first stages of disease, fungal hyphae have not frequently been observed, in association with discoloured tissues. In a few cases oospores indicating the presence of pythiaceous fungi were observed in or in connection with discolouring tissues (cf. FRITZSCHE and VOGEL, 1954; MULDER, 1968). As decay advances, several secondary organisms can be seen in the tissues, including saprozoic nematodes. Bacteria and actinomycetes, however, are always present also on apparantly healthy roots. With beginning discolouration they can be found on the surface of the roots, and inside the epidermal cells including root hairs, and less often in cortical layers.

Inside the stele no abnormalities have been observed other than in an advanced stage of decay, indicating that only secondary organisms were involved. Parenchymatous tissues here often contain starch. This is also true for older roots in which no abnormalities were seen.

Summarizing, the symptoms suggest that the causal factor of SARD attacks the root system rather superficially, and also, that the disease may be connected with processes normally occurring in, on, or around feeder roots of healthy trees, but developing more strongly or rapidly in the case of disease. Epidermis and cortex are tissues essential for normal functioning of the primary root. This may explain the rather strong growth reduction resulting from the general decay of these tissues.

As already stated other points can be mentioned which make it possible to characterize SARD more sharply than by its symptoms alone (cf. SAVORY, 1966):

The specificity, as implied by the name of the disease, is one of its most striking characteristics. In the Netherlands, all important agricultural crops are known to grow well on apple soil. In a pot experiment, various plants including barley, grass, corn, carrots, tomatoes, beans, peas, and lettuce were found by us to grow very well on apple soil. In heavily infested orchards, yields of potatoes and beets were reported by growers to be higher than on average arable land. These crops apparently take full profit of the good structure and high fertility of former orchard soils. The disease is also specific within the group of fruit species. Apple can safely be replanted on cherry soil and cherry on apple soil.

TABLE 4. Pot experiment with apple and cherry seedlings, planted on untreated, DD treated and CP treated apple and cherry soil. Indication of significance of differences with growth on untreated apple soil for apple seedlings, and on untreated cherry soil for cherry seedlings.

Soil	Treatment	Apple seedlings (cm)	Apple seedlings (%)	Cherry seedlings (cm)	Cherry seedlings (%)
Apple	Control	5.6	100	15.0**	100
	DD	6.9	123	20.6**	137
	CP	17.1**	305	19.4**	129
Cherry	Control	12.5*	100	4.7	100
	DD	15.3**	122	5.3	113
	CP	18.9**	151	16.7**	355

Table 4 illustrates the specificity of apple and cherry replant disease with data from a pot experiment. With regard to pear the situation is less clear, and there is also less information available. There is at least specificity to pome fruit. When considering this problem, it should be realized, that not only specificity, but also susceptibility and degree of disease induction by different species may play a role.

The causal factor of the disease is very persistent in the soil. VAN MARLE (1962) found no differences between replantings immediately after grubbing, and after periods up to five years (the maximum period in his investigations). The persistence was also observed in field experiments performed at different intervals after grubbing, irrespective of intervening crops. SCHANDER (1956) quotes the observation that, in Germany, Asparagus is considered a particularly favourable preceding crop for apple. Pot experiments with apple soils in which fresh Asparagus roots were mixed into the soil, and Asparagus was grown for six months, did not show any effect of these treatments.

An interesting case illustrating both persistence and specificity was reported by THOMPSON (1959). A field was used as a nursery from 1941–1953, then cultivated for 5 years with other crops (wheat, seed cocksfoot grass, and potatoes) and in 1958 planted again with various nursery crops. The rows of the second planting of nursery crops were at right angles to the rows of the original planting. In the second planting areas of poor growth appeared where closely related species were grown before, especially in the case of apple and cherry. Quince was also affected to some degree after apple. This last observation confirms that the specificity may concern groups of related species rather than single species.

In a pot trial with soil from a field experiment in the river clay area cultivated for three years with M. IV apple rootstocks and Quince A, we observed that growth of apple seedlings was equally poor after both species. Some of the data of VAN MARLE (1962) also indicate that apple may grow poorly after pear.

Another characteristic of SARD is recovery after transplanting to fresh land. PITCHER (fide SAVORY, 1966) showed quick recovery of one year old apple and cherry nursery stock. After one year, the transferred plants outstripped previously healthy plants which had been simultaneously transferred into soil affected by the respective replant diseases.

With apple trees, various typical cases of recovery after transplanting to fresh land were observed on Dutch fruit farms. A special case of recovery occurred with apple seedlings in some of our greenhouse experiments: stunted seedlings from apple soil were transplanted to aerated water cultures. Root growth was resumed within three days, and vigorous shoot growth within seven days.

Apart from the data already quoted, SAVORY (1966) gives more information from the literature, illustrating these characteristics with apple as well as other crops. From his publication it is clear that the characteristics: specificity, persistence and recovery after transplanting to fresh land can all be observed with most cases of specific replant diseases discussed. It is also suggested, that the absence of clearly pathogenic organisms is a common character of specific replant diseases. It is doubtful, however, if this characteristic can be maintained upon closer observation and study of groups of organisms so far not recognised as possible causes of this type of diseases. In the case of cherry replant disease for instance, it was shown that *Thielaviopsis basicola* (Berk. & Br.) Ferr. is a factor (HOESTRA, 1965) This fungus is not pathogenic to apple.

Persistence and reversibility are characters which show that SARD is very clearly connected with the soil. A further discussion of the significance of symptoms and characteristics will follow in chapter 5 (general discussion). It should only be added here that the two characteristics quoted above also apply to damage by *Pratylenchus penetrans*. The main points in which the nematode disease differs from SARD are: 1. the presence of the nematode can easily be demonstrated in the roots, 2. there is a more patchy distribution of poorly growing trees over the field, 3. damage by the polyphagous nematode is not restricted to apple and closely related crops.

3.2. Experimental demonstration

It is obvious that for both scientific and practical purposes a means of demonstrating SARD in the soil is extremely important. Since the cause of the disease is unknown, it was tried to find an indirect method.

Since both apple trees and seedlings are susceptible attempts were made to demonstrate SARD by means of pot experiments. An example has already been given of specificity of apple and cherry replant diseases in a pot experiment with seedlings under greenhouse conditions (table 4). Pot experiments already in the first stage of this work showed poor growth of seedlings on apple soil and good growth on fresh soil. This indicated that SARD could be reproduced in pot experiments.

As a consequence of these first results a larger series of pot experiments was carried out in 1959–1960. Large size pots (10 l) and Malling type IV rootstocks were used.

The experiment included 10 soil samples, and 11 treatments. A full account of this experiment will follow (4.2.). Here 8 samples from the Bangert area near Hoorn, Province of North-Holland, and those treatments which illustrate reproduction of SARD in the pots will be dealt with. The figures show that growth in untreated soil was much better on fresh than on apple soils (table 5).

TABLE 5. Pot experiment with 6 apple soils and 2 fresh soils from the Bangert area. Average shoot growth of rootstocks M. IV in the first year after planting, and averages of effect of treatments in % of controls.

Treatment	Apple soil		Fresh soil	
	(cm)	(%)	(cm)	(%)
Control	16	100	40	100
Nitrogen	17	112	50	125
DD	28	179	56	145
CP	53	353	62	159
Heat (60°)	47	309	60	150
Heat (120°)	51	335	56	144

All apple soils came from fields which did not offer any problem as to growth for other crops besides apple. In most cases the nematode figures were low,

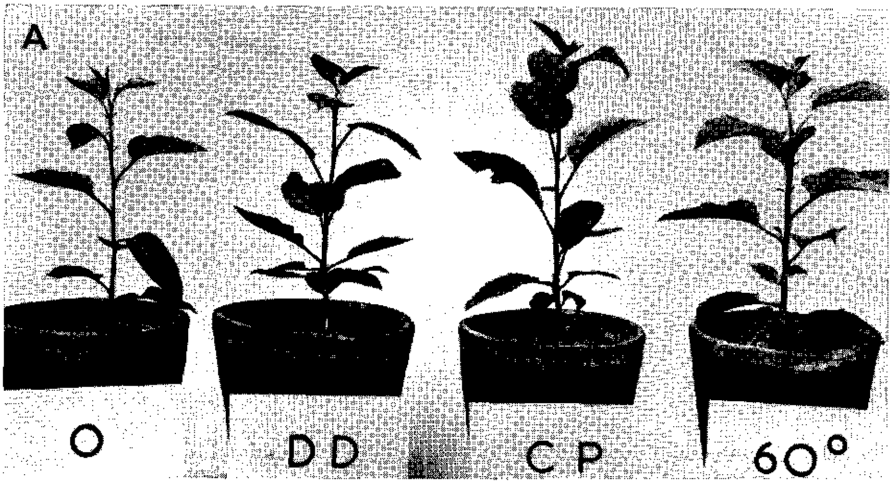


FIG. 5a

and the effect of the nematicide DD was moderate. It was concluded that the differences in growth between the fresh and the apple soils were due to SARD. The broad spectrum treatments with chloropicrin and heat were all about equally effective in improving growth on apple soils. The nitrogen treatments had a small effect. This makes it plausible to conclude that growth improvement after broad spectrum treatments is not the result of the so called nitrogen effect (see also section 4.2.). It was concluded that the broad spectrum treatments were able to control replant disease. These treatments – without sterilizing the soil completely – kill the major part of soil organisms of most groups.

It thus appeared, that under the conditions of the pot test SARD can be demonstrated by the difference in growth between controls and plants on soil which is disinfected by broad spectrum treatments. This conclusion also suggested to test these treatments in smaller pots, in further work on the reproduction of SARD. These investigations also proved successful; SARD is reproduced well in experiments with pots of 500–750 ml (figure 5). The experiments to be described in section 3.3. were all carried out in these types of pots, and in section 4.6. a detailed discussion will be given of the application of the pot test for advisory work.

Theoretically it would also be possible to compare growth on fresh and apple soils in a pot experiment to test for SARD. In many cases it would be very difficult, however, to find a sample of fresh soil which is not differing from the sample of apple soil, with regard to general fertility, structure and infestation with organisms not related to SARD.

The use of broad spectrum soil disinfectants also presents problems which require some precautions. Firstly in order to avoid the nitrogen effect to influence the experimental results appreciably, a high level of nitrogen is desirable in all treatments. This has been realized by applying a fertilizer mixture rich in nitrogen. Secondly to help to determine the effect of nematodes present it is

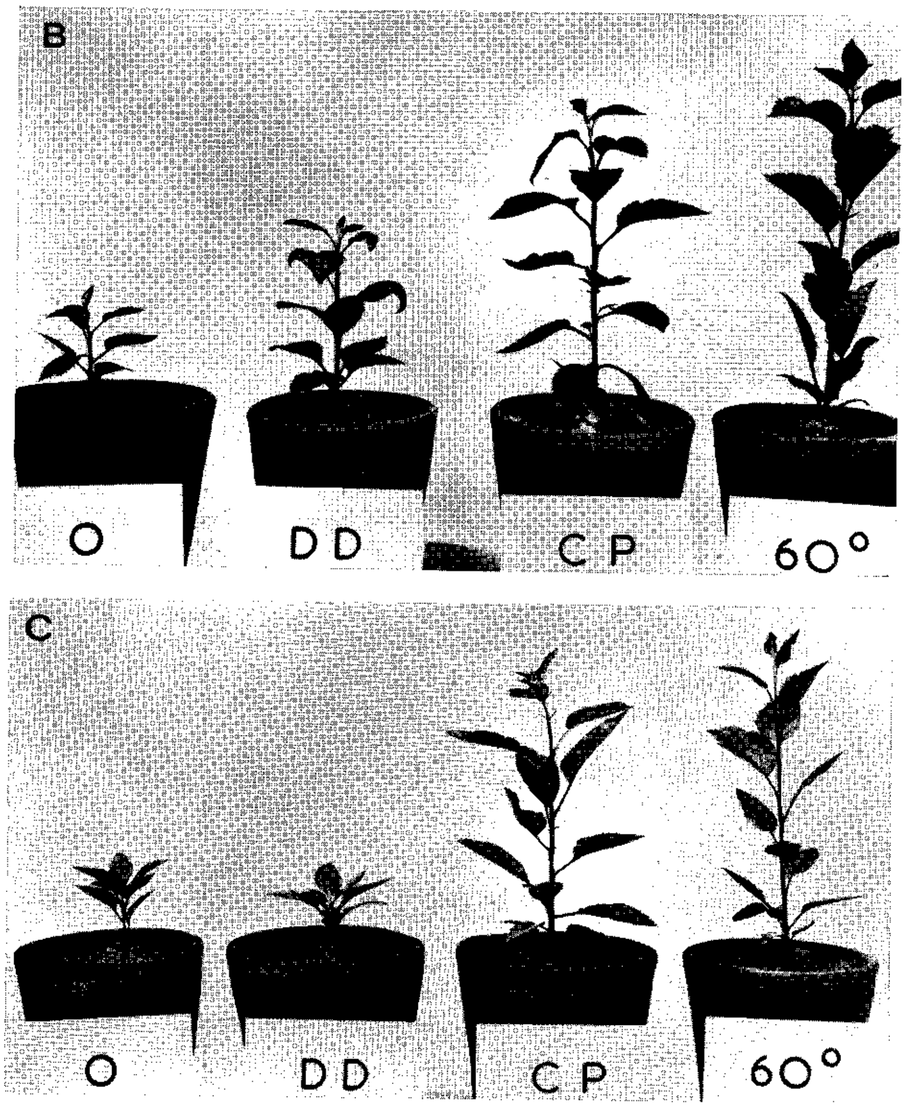


FIG. 5. Effect of DD, CP and heat treatments (60 °C, 2 hours) of soil on growth of apple seedlings. A: Fresh soil, B and C: Apple soils.

advisable to include a treatment with a specific nematicide. In almost all experiments DD was used for this purpose. Since DD also incites a nitrogen effect its application offers a second check on the nitrogen effect induced by CP.

3.3. Experimental characterization, effect of various treatments

3.3.1. *Introduction*

In the two foregoing sections data were presented which on the one hand form a sufficiently firm basis for research on practical aspects; and on the other hand led to investigations, to be described in this section, into a further characterization of SARD, in order to get a better understanding of its background and cause. For this purpose emphasis was laid on attempts to transmit the disease or reproduce symptoms, and on studies on the effect of conditions. The need for this type of work was felt strongly also because attempts to determine the cause of SARD by direct methods, failed. Observations of symptoms did not lead to conclusions about the causal factor, and inoculation experiments with a range of fungi isolated from apple roots and surrounding soil did not yield evidence of pathogenicity of any of these species. These investigations, including field observations and pot experiments, were carried out throughout the duration of the research programme. The pot experiments were based on the observation that SARD is reproduced under the conditions of these tests, as will further be substantiated in section 4.6.

In most cases use was made of soil taken from field experiment no. 6, which was strongly infested with the causal factor of SARD, and only lightly with plant parasitic nematodes. Another reason for the choice of this soil was its good and stable structure. In the tables concerned the soil is indicated as 'M' soil, after the village of Meteren in the river clay area, where the field was located. In addition to the information on backgrounds of the disease some of the experiments gave results of practical interest. Brief conclusions on the observations and experiments are given but the main discussion on backgrounds and possible causes will follow in chapter 5: General Discussion. This procedure is preferred, because facts contributing to our knowledge on backgrounds of the disease also came from experiments on control (chapter 4). Yet it is of interest here to indicate that with regard to the cause of SARD, 4 main groups of possibilities will be considered in the general discussion, and these were also kept in mind when designing the experiments to be described. These four groups are: physical soil properties, deficiencies, toxins and organisms.

3.3.2. *Depth of occurrence in the soil*

For several reasons it is important to know the vertical distribution of SARD. Practical aspects are to what depth soil samples have to be taken and treatments for control have to be made. From the scientific standpoint knowledge on the depth of occurrence may contribute to an understanding of the backgrounds of the disease.

Samples have been taken at different depths in apple orchards known to be infested with SARD. The stimulation of growth by chloropicrin was used as an indication of disease intensity. Table 6 gives the results. The figures indicate that generally speaking disease intensity decreases below 30–45 cm. The maximum effect of CP treatments is found in the 0–15 and 15–30 cm layers. Soil from

TABLE 6. Effect of depth of sampling on growth of apple seedlings and on CP effects. Pot experiments with soil from 3 apple orchards. Soils nos. 1 ('M') and 2 are from the river clay area, no. 3 is from the Bangert area.

Depth (cm)	Growth on untreated soil (cm)			Growth on CP treated soil (% of controls)		
	1	2	3	1	2	3
0–15	5.9	3.9	7.9	357**	269**	177**
15–30	5.9	3.4	7.0	324**	232**	210**
30–45	6.1	2.5	8.8	209**	211**	141
45–60	6.1	2.9	10.7	140	170	116
60–75		3.7			147	

these layers should therefore be used in pot tests. Treating soil in the field can generally be confined to the first 50 cm, especially since SARD mainly affects trees in the first year after planting, when they depend on the superficial layers of the soil.

Decreased disease intensity in deeper layers may be related to decreased intensity of rooting, but in all these cases roots were present in the deepest layers sampled. It seems more probable that the differrences in conditions in the different layers influence the causal factor.

3.3.3. *Leachates from apple soils*

Adding leachates of apple soil to healthy soil has been reported by FASTABEND (1955) to induce growth reduction though growth was still somewhat better than on apple soil. In our experiments this treatment led to variable results, but never to strong growth reductions. Interpreting the results of this type of experiments is difficult. The repeated addition of leachates may lead to the accumulation of chemical compounds and organisms which may have nothing to do with the disease, but can have effects beyond those in normal concentrations.

In one experiment portions of 500 ml of apple soil were placed in glass tubes of 40 cm length and leached 10 times during a period of 6 weeks, each time yielding 50 ml of soil leachate per tube. The liquid was administered immediately to pots of steam sterilized apple soil, 50 ml soil per pot each time. After the 6 weeks period the pots were planted with seedlings. As the soil in the tubes was moist during the percolation period a treatment was included in which apple soil was kept saturated with water during the 6 weeks percolation period. The

TABLE 7. Pot experiment with apple seedlings. 'M' soil. Effect of leaching soil and additional treatments.

Control (cm/%)	Leached (%)	Kept wet during leaching period (%)	Steamed (%)	Steamed, leachates from untreated soil added (%)
3.5/100	129	123	267**	231**

results show (table 7) that no appreciable growth reduction resulted from the addition of soil leachate, and also, that the leached soil did not loose its capacity of reducing growth of apple seedlings. From this experiment it appears that the causal factor of SARD is not readily transmitted with soil leachates.

3.3.4. *Nutrient solutions from mist cultures of apple*

Plants can be grown in mist cultures; with this system, the roots are constantly sprayed with a fine spray of nutrient solutions. The I.B.S. at Wageningen developed this technique for growing apple trees. The root systems are placed in 200 l drums and exposed to the nutrient solutions which circulate for several weeks before being refreshed. In this way normal development of trees could be obtained in the greenhouse, and even fruit production in mid-winter.

Two experiments were done to test the possible action of these solutions on apple seedlings[1]. In one experiment two groups of 12 seedlings each were grown, one group in a closed system of fresh nutrient solution, and the second in a solution which also circulated over the root systems of two apple trees. All plants were growing well. No evidence of SARD was present. Formation of side branches was somewhat more luxurious with the fresh solutions than with apple solutions, but this was probably the result of competition for food in the latter. This factor could not entirely be excluded in this case. In another experiment nutrient solutions from mist cultures of apple which had circulated for two months, were applied to pots of seedlings in the greenhouse. Two soils were used, a steam sterilized apple soil, and a fresh soil rich in organic matter. With both soils, half of the seedlings received solutions without further treatment, and the other half solutions heated to 100°C for 30 minutes. This heat treatment, effectively controlling SARD in the soil (section 4.4.8.) could be supposed to eliminate the causal factor. The solutions were administered regularly during the first six weeks after planting. Growth was normal in the two treatments of either soil.

3.3.5. *Apple roots*

The data available on the effect of adding apple roots to soil are somewhat contradictory. FASTABEND (1955) found a strong growth reduction by adding 1 % of crushed apple roots to the soil. BÖRNER (1960) did not observe any growth reduction with 4 % of apple roots. Similar results were obtained by COLBRAN (1953). In England it was concluded that root residues are not harmful to the replanted trees (SAVORY, 1967). Some growth reduction was reported when roots were added to fresh soil, and a stimulation when added to apple soil (ANONYMOUS, 1967b). The same results were obtained in a preliminary experiment at Wageningen. In another experiment apple roots (2 %) both fresh and heat treated, were added to 10 different soils, including two fresh soils. Growth of Malling IV rootstocks was slightly stimulated in most cases, especially when fresh roots were used (see table 17 in section 4.2). These results indicate that the causal factor is not transmitted by adding apple roots to the soil. The

[1] Thanks are due to Dr. J. Doeksen of the I.B.S. for his help in realizing these experiments.

cases of growth reduction by adding roots to fresh soil, quoted above, may well be explained by temporary nitrogen deficiency occurring with the decomposition of the root tissues. The stimulating effect of adding roots to apple soil is particularly interesting because this suggests that the causal factor may be suppressed to some degree by apple root residues.

3.3.6. *Phloridzin*

Phloridzin, a glucoside, is an important constituent of the apple tree, notably of the root cortex. It has been supposed to play a role in apple replant disease since 1959 (BÖRNER). In water cultures it had a strong reducing effect on growth of apple seedlings. This effect could not be reproduced when phloridzin was added to soil (BÖRNER, 1961) or sand (ANONYMOUS, 1962b).

In one pot experiment in which perlite was used instead of soil, 2 g of phloridzin per 500 ml pot had no effect on growth of apple. In a second experiment phloridzin was added to untreated and steam-sterilized apple soil, and to a mixture of the two (20% untreated soil). Also N treatments were included alone and in combination with phloridzin, because it was supposed that the breakdown of phloridzin could be affected by the nitrogen level (table 8).

TABLE 8. Effect of phloridzin treatments on growth of apple seedlings in pot experiment ('M' soil).

Soil	Treatment	Dosage (g/l of soil)	Length of seedlings (cm)	Length of seedlings (%)
Apple soil	Control		3.6	100
	Phloridzin	5.0	4.3	119
	NH_4NO_3	0.5	4.0	111
	Phloridzin+NH_4NO_3	5.0+0.5	5.4	150
Steamed apple soil	Control		12.2	100
	Phloridzin	5.0	12.5	102
	NH_4NO_3	0.5	12.1	100
	Phloridzin+NH_4NO_3	5.0+0.5	12.0	98
Apple soil 20%	Control		6.5	100
Steamed apple soil 80%	Phloridzin	5.0	8.7*	134*
	NH_4NO_3	0.5	6.1	94
	Phloridzin+NH_4NO_3	5.0+0.5	9.4*	145*

Phloridzin and NH_4NO_3 were applied 4 weeks before planting at the rate of 5 and 0.5 g/liter of soil respectively.

Under the conditions of this experiment phloridzin has not in the least suppressed growth of apple seedlings. It is, on the contrary, clear that phloridzin has stimulated growth, especially when applied to the mixture of steamed and diseased soil.

3.3.7. *Mixing soils*

In a pot experiment, a series of mixtures of apple soil and steam-sterilized apple soil, and of apple soil and fresh soil was prepared. The results are shown

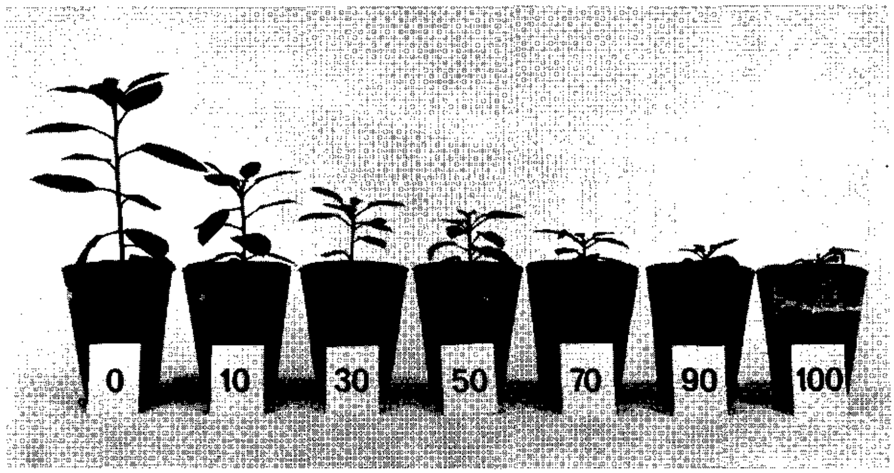


FIG. 6. Effect of mixing untreated and steam-sterilized apple soil on growth of apple seedlings. From left to right: 0,10,30,50,70,90 and 100% apple ('M') soil.

TABLE 9. Pot experiment with apple seedlings. Effect of mixing steam-sterilized apple ('M') soil, and fresh soil, with different proportions of apple soil. Effects on length and weight indicated in % of growth on steamed apple soil and fresh soil respectively. Significance of results applies to differences with growth on steamed and fresh soil respectively.

Proportion of diseased soil	*Steam sterilized apple soil* length (*cm* and %)	fresh weight (*g* and %) tops	fresh weight (*g* and %) roots	top/root ratio (%)	*Fresh soil* length (*cm* and %)	fresh weight (*g* and %) tops	fresh weight (*g* and %) roots	top/root ratio (%)
0	*20.7*	*7.4*	*11.4*	65	*21.0*	*7.3*	*15.4*	47
	100	100	100		100	100	100	
10	54**	58**	84*	45	75**	77*	101	36
30	40**	35**	45**	51	52**	55**	60**	43
50	34**	43**	55**	51	33**	32**	34**	44
70	32**	39**	39**	64	29**	32**	26**	58
90	27**	31**	34**	59	22**	25**	21**	55
100	23**	15**	18**	52	25**	26**	25**	49

in fig. 6 and table 9. As was also observed in two other experiments of the same type it is clear that 10–20% of untreated apple soil leads to a marked growth reduction, but growth in these series of treatments is still clearly better than on 100% apple soil.

The reduction of growth by adding 10% of infested soil is stronger with the steam-sterilized apple soil than with the fresh soil. It may be supposed that the former soil is a more favourable medium for the establishment of the causal factor than the latter. It should be added, that the steam sterilization took place 4 weeks before the mixtures were prepared, and that, therefore, recolonization with saprozoic organisms will have been well under way in the steamed soil when it was mixed with the untreated apple soil.

3.3.8. *Soil moisture content*

Soil moisture content is an essential factor in host-pathogen relationships and influences the degree of disease incidence of many soil - borne diseases. SCHANDER (1958) observed that SARD is less serious in fields with high water tables; in the Netherlands similar observations were made. An experiment was therefore done to test the effect of soil moisture on growth of seedlings in untreated and CP treated apple soil.

There were three levels of soil moisture content maintained by weighing and watering pots three times a week. To reduce evaporation by the clay pots, plastic bags were put inside the pots. The results are shown in table 10. It is obvious

TABLE 10. Pot experiment with apple seedlings. Effect of soil moisture content on growth of apple seedlings on CP treated and untreated 'M' soil. At each level of soil moisture content CP effects were significant, at 1 or 5% level. Soil moisture effects were significant in the following cases (differences with growth on the soil with the lowest moisture content of the same treatment): Length and dry weight of tops: CP (37.4), CP (31.6), 0 (37.4). Dry weight of roots: CP (37.4).

Soil moisture content (% of dry weight)	Fumigation treatment	Length (cm)	CP effects (length, %)	Dry weight (g)		CP effects (total dry weight, %)	Top/root ratio (%)
				tops	roots		
25.8	O	5.5		1.02	0.85		120
	CP	13.5	245	4.13	2.11	334	196
31.6	O	9.5		2.79	1.43		195
	CP	19.1	201	7.31	3.63	259	201
37.4	O	14.5		5.44	2.31		235
(field capacity)	CP	25.9	179	11.36	6.36	229	179

that the effect of moisture content is very marked. Both growth in control and CP treated soil is stimulated by high moisture content, but this effect was most pronounced in the controls. Relatively the growth improvement by CP, as compared with the control, is therefore strongest at the lowest moisture level, though the differences in the ratio of the two treatments are much less important than the differences due to the moisture content. The main conclusion of this experiment is that in pot experiments differences in moisture content between treatments can interfere strongly with the results of these treatments and have to be avoided. Therefore in pot experiments use has been made of a tensiometer. Still in the greenhouse in certain experiments differences between treatments have existed, at least temporarily, in so far that soil was somewhat dryer in pots in which plants grew well than in the controls. In these cases the effect of treatments stimulating growth are likely to be somewhat underestimated. With pots buried in the soil the problem is less serious because the surrounding soil will tend to level out differences in moisture content in the pots. With regard to the effect on disease, the experiment suggests that SARD is more serious in relatively dry soils.

3.3.9. *Soil temperature*

To study the effect of soil temperature on disease incidence, an experiment was carried out in Wisconsin tanks with temperatures ranging from 15 to 35 °C[2]. The results (table 11) show that appreciable differences between CP treated soil

TABLE 11. Pot experiment with apple seedlings in Wisconsin tanks. Effect of soil temperature on growth of apple seedlings on untreated and CP treated 'M' soil. At each soil temperature CP effects are significant, at 1 or 5% level, except for dry root weight at 35 °C.

Soil temperature (°C)	Fumigation treatment	Length of of seedlings (cm)	CP effects (length, %)	Dry weight (g)		CP effects (total dry weight, %)	Top/root ratio (%)
				Tops	Roots		
15	O	9.6		1.29	0.64		202
	CP	23.2	242	4.01	1.77	299	227
20	O	7.2		0.78	0.51		153
	CP	20.4	283	3.01	1.59	357	189
25	O	5.4		0.49	0.19		258
	CP	15.4	285	2.26	0.60	421	377
30	O	8.8		0.97	0.24		404
	CP	24.2	275	3.36	1.14	372	295
35	O	8.1		1.18	0.37		319
	CP	14.4	178	2.12	0.43	165	493

and the controls occurred at all temperatures, suggesting that the causal factor is able to reduce growth of apple seedlings at all these temperatures. The top/root ratio is affected both by the temperature and the CP treatment. At 35 °C the root system remained very small, also in CP treated soil. The difference between CP and the control in this case may partly be the result of increased susceptibility as can be expected to occur with such reduced root systems. A lower level of infestation with the causal factor of SARD would then not result in a better growth of the controls.

In two experiments with healthy apple seedlings in watercultures, performed by Dr. Brouwer of the I.B.S., Wageningen, it was shown that seedlings grew well without much differences, with root temperatures ranging from 15–30 °C. At 10 °C, and below, a marked growth reduction was observed.

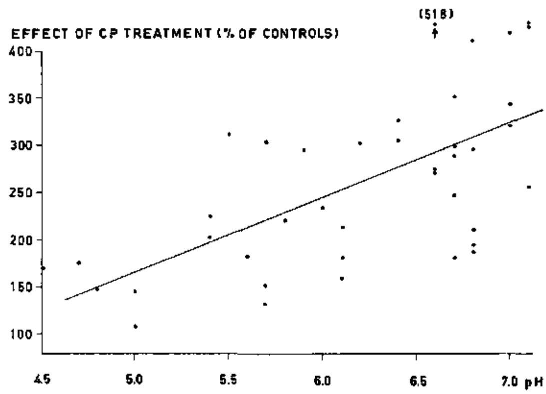
3.3.10. *Soil pH*

The effect of pH – an important factor in many soil-borne diseases – was

[2] I thank Ir. W. P. de Leeuw, of the P.D., Wageningen, for providing facilities for carrying out this experiment, and for helpful suggestions.

first observed in a series of pot experiments in 1962 and 1963 which served to test the suitability of a pot experiment as a basis for advisory work. In all cases in which very severe replant disease occurred pH was high. There were smaller numbers of samples with low pH, but, in none of these severe replant disease occurred (fig. 7). In later years, when the pot test was applied at the 'Bedrijfslaboratorium' as a routine test, the same effect was observed, though it was not always very marked. Apparently other factors also influence the occurrence of replant disease. Cases where infestation was nil or only slight were also found at high pH values.

FIG. 7. Relation between pH of apple soils and effect of CP treatments on growth of apple seedlings in pot experiments.



In pot experiments the effect of pH was further tested (HOESTRA and KLEIJBURG, 1967). In the first, H_2SO_4 and $Ca(OH)_2$ in different dosages were used to make a series of widely differing pH levels. After the first crop of seedlings the experiment was continued by growing a second crop without further treatment (Table 12).

TABLE 12. Pot experiment with apple seedlings. Effect of pH of soil. Final length of seedlings in two successive plantings ('M' soil).

Treatment	Dosage (per l of soil)	pH (start of 1st planting)	Length of seedlings, 1st planting (*cm* and %)	pH (start of 2nd planting)	Length of seedlings, 2nd planting (*cm* and %)
Control		6.45	*5.5*	6.7	*3.9*
			100		100
Chloropicrin	0.2 ml	6.4	251**	6.6	185*
$Ca(OH)_2$	2.5 g	6.8	93	7.2	108
$Ca(OH)_2$	5.0 g	7.05	96	7.3	87
$Ca(OH)_2$	7.5 g	7.4	105	7.4	74
H_2SO_4(95%)	1.85 ml	6.1	135	6.4	162*
H_2SO_4(95%)	3.70 ml	5.9	173	6.1	182*
H_2SO_4(95%)	5.55 ml	-	244**	5.8	356**

In another experiment of the same type 4 crops of apple seedlings were planted in periodes of 4–5 months each. Sulphur treatments were included. The dosages and pH values are shown in table 13 and the effects on growth of seedlings in figure 8.

TABLE 13. Effect of different treatments on pH of soil in pot experiment planted four times in succession with apple seedlings ('M' soil).

Treatment	Dosage (per l of soil)	pH at first planting date, 8 weeks after treatment	pH at the end of plantings 1	2	4
O		6.2	6.9	6.9	6.9
CP	0.2 ml	6.4	6.5	6.6	6.7
$H_2SO_4$95%	10 ml	5.2	5.7	5.6	6.0
Sulphur (S_1)	5.7 g[1]	6.0	5.8	5.5	5.6
Sulphur (S_2)	11.4 g	5.9	4.3	3.9	4.3

[1] Equivalent to 10 ml $H_2SO_4$95%

The experiments confirm that low pH suppresses the disease. Sulphur as such is not effective in controlling the disease, as it is clear that the treatments only stimulate growth when pH is decreased as a result of the activity of sulphur oxidizing bacteria. The results with sulphur also indicate that H_2SO_4 effect is not based on a sterilization of the soil as might be supposed to occur when the strong acid is added. The same conclusion could be drawn from other experiments in which soils with high calciumcarbonate contents were treated. In these experiments H_2SO_4 did not lower pH and neither did improve growth. The results shown in figure 8 and tables 12 and 13 also indicate that low pH decreases the development of the causal factor in the soil, because in the CP series, which showed strongly improved growth in the first planting, the causal factor apparently re-established itself in subsequent plantings, while in the series with lowered pH it did not to that extent.

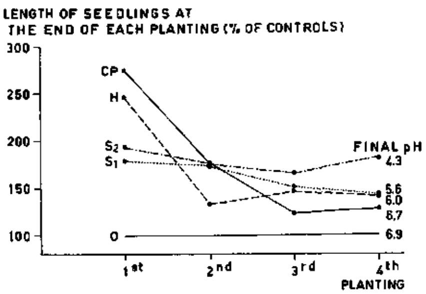


FIG. 8. Effect of treating apple soil with CP, H_2SO_4 (H) and sulphur (S) on growth of four subsequent plantings of apple seedlings in a pot experiment ('M' soil).

The beneficial effect of lowering pH by H_2SO_4 was confirmed in many cases. In carrying out this treatment at the 'Bedrijfslaboratorium' in all samples sent in for advice on SARD in 1967, the standard dose of 10 ml H_2SO_4 95% per l of soil was too high for a number of soils and seedlings died. Very good growth was possible at pH 3.8 and higher, which is in agreement with results by EDGERTON (1940). The effect on growth was relatively small if, with high initial values (7 or higher) pH was decreased by 0.5 or less.

In these cases the effect of chloropicrin was generally better than that of H_2SO_4. When pH is lowered by more than 0.5, there is a good correlation between H_2SO_4 and CP treatments, if cases of final pH values below 3.8 are not considered.

These observations confirm that the stimulating effect of H_2SO_4 is indeed the result of lowering pH. In field experiments sulphur treatments may lead to lowering pH from three months after application in early spring, depending on initial pH. Growth differences on trees have so far not been observed. The experiments are still under way. Soil acidification seemed to have a favourable effect on soil structure, making it friable and easy to work as compared with the control plots.

An interesting side effect of pH is a difference in colour of the young suberized root system between plants grown in soils of pH 6 or lower, and in soils of higher pH. The latter have a characteristic reddish brown colour, while roots from low pH soils are definitely lighter coloured, mostly being pale-yellow. This was observed both in H_2SO_4 treated soils, and in soils with a natural pH below 6–6.5.

In conclusion it can be said, that by various series of observations and experiments low soil pH has been shown to prevent the occurrence of SARD, and that lowering pH of near neutral apple soils has a curative effect. Both aspects were also observed in England (ANONYMOUS, 1967b; SAVORY, 1967). Serious difficulties in replanting apples are reported from Spain on high pH soils, while less serious problems occur on soils of lower pH in Poland and Germany (Personal communications by BOLIVAR, SLOWIK, and LOEWEL, respectively).

3.3.11. *Gibberellic acid*

A symptom of severe replant disease of apple is rosette formation with many small leaves around the final bud. Obviously, arrested stem elongation is a component of this symptom. Therefore the effect of gibberellic acid which is known to be an effective stimulator of stem elongation with apple and other plants has been tested. Soil was used from a pot experiment with plants showing symptoms, and replanted with young seedlings in the 2-leaf stage. Gibberellic acid (G.A. 7) treatments started 4 weeks after planting when growth had stopped. One drop of a 100 ppm solution was placed as near to the bud as possible on each plant 5 times per week over a period of three weeks. Gibberellic acid caused the plants with dormant buds to resume growth two or three days after the first application. The plants continued to grow, but were very poor; leaves were small, narrow and light coloured. The treatment obviously did not produce normal plants; instead, the seedlings were only 'normal' in length, but poor in every other respect.

Similar results were obtained in other experiments; it appeared that older seedlings, which had ceased to grow for periods up to 4 months before the treatment started, could also be easily forced to resume growth. The newly formed shoots, too, were far from normal, showing the same type of poor narrow leaves.

These observations indicate that poor growth of seedlings is not only the result of inhibition of stem elongation, but is of a more general nature, as would be expected in cases where the condition of the root system is poor.

3.3.12. *Summary of results*

The causal factor of SARD is not present to an appreciable degree below 45 cm in the soil, and is markedly suppressed by low soil pH. High soil moisture content stimulates growth of apple seedlings strongly in both CP treated and untreated apple soil, but strongest disease intensity was observed in the case of the lowest moisture content. Soil temperature had no effect in the range of 15–30°C. In mixing soils the presence of 10% of diseased soil reduced growth of apple seedlings appreciably, but growth was still markedly better than on 100% apple soil. In attempts to transmit or induce the disease no results were obtained with leachates from apple soils, nutrient solutions from mist cultures of apple trees, apple roots or phloridzin. On the contrary, with the latter two treatments a growth stimulation was observed when applied to apple soil. Experiments with gibberellic acid showed that the effect of SARD is not restricted to inhibition of stem elongation.

4. CONTROL OF SPECIFIC APPLE REPLANT DISEASE

4.1. Introduction

In this chapter, research on control measures is described. This work comprises testing the effect of various treatments, (sections 4.2.–4.4.) and development of an advisory method based on pot tests (bioassay) described in section 4.6. Many of the experiments were also useful to gain more insight in the backgrounds of the disease, and certain treatments were especially included with this in mind.

The pot experiment already mentioned in 3.2. was carried out in the first stages of the work and was important because it gave information which was essential for the planning of the further work (4.2.). Another reason for its discussion in a separate section is, that this experiment was the only one in its kind in which large pots (10 l) were used.

Field experiments, discussed in 4.3., are summarized per region, to emphasize the situations with respect to SARD in the different fruit growing centres.

The experiments described in 4.4. have been carried out with 0.5 l pots and seedlings as the test crop, in the greenhouse. They are discussed per group of related treatments.

Table 14 gives information on the compounds used in the experiments on control.

Conclusions on the two sections, 4.3. and 4.4. will be given in section 4.5.

4.2. Exploratory pot experiment

In this experiment 10 different soils were used. Eight of these, 6 apple soils (nos. 1–6) and 2 fresh soils (nos. 9 and 10), had been taken from the old fruit growing centre de Bangert and surroundings in the Province of North Holland.

One soil was from an apple orchard in the south western part of the country (7), and another from an apple nursery on sandy peat soil from the north (8). The samples were taken in September–November 1959. Heat and fumigation treatments followed in winter. For fumigation treatments the soil was placed in 30 l milkchurns. Other treatments included application of fungicides, ammoniumnitrate, and apple roots; these materials were mixed into the soil shortly before planting. The pots were buried into the soil, in the open shortly before planting. The treatments are shown in table 17. There were 5 replicates. In each pot three one-year-old Malling IV rootstocks were planted of 6–8 mm diameter.

Table 15 shows some general characteristics of the soils and reveals no important differences between fresh and apple soils.

In most samples numbers of parasitic nematodes were low at the beginning of the experiment (table 16). In the case of the apple soils the highest numbers of nematodes in most genera occurred in soils 3 and 6. This can be explained by the presence of a grass cover in the two orchards concerned.

TABLE 14. Chemical compounds, and mixtures of chemical compounds, used in experiments on control of SARD. Trade names, and code numbers are placed between quotation marks, abbreviations between brackets.

Common name, trade name, code number, abbreviations.	Chemical name, composition of mixtures
Allyllidene diacetate	2-propene-1, 1-diol diacetate
'Aretan'	methoxyethyl mercuric chloride
Benquinox	quinone oxime benzoylhydrazone
Captan	N-trichloromethylthiotetrahydrophthalimide
Chloropicrin (CP)	trichloronitromethane
(CBP)	3-chloro-3-bromo-1-propene
Dazomet	3,5-dimethyl-1.3.5.2 H-tetrahydrothiadiazine-2-thione
'Dexon'	p-dimethylaminobenzenediazo sodium sulfonate
(DD)	mixture of 1,2-dichloropropane 1,3-dichloropropene and related hydrocarbons
'Ditrapex'	mixture: MIT 22%, dichloropropene 51%
Ethylene dibromide	1,2-dibromo ethane
'EP 201'	mixture: dichloropropenes, dichloropropane and related hydrocarbons 68%; MIT 17%; CP 15%.
Formalin	formaldehyde
Metham-sodium (metam)	sodium N-methyldithiocarbamate
Methyl bromide	monobromomethane
(MIT)	methyl isothiocyanate
Nabam	disodium ethylenebisdithiocarbamate
Propylene oxide	1,2-epoxypropane
Quintozene	pentachloronitrobenzene
'Rhizoctol'	methyl arsenic sulfide
(TCTNB)	1,3,5-trichloro-2,4,6-trinitrobenzene
Tecnazene	1,2,4,5-tetrachloro-3-nitrobenzene
'Telone PBC'	mixture: 1,3-dichloropropene 80%; CP 15%; 3-bromopropyne 5%.
'Temik'	2-methyl-2-(methylthio)-propionaldehyde-O-(methyl-carbamoyl) -oxime
Thiram	tetramethylthiuram disulfide
'Tridipam'	N N[1]-dimethylthiuram disulfide
'Trizone'	mixture: methyl bromide: 61%; CP 30%; 3-bromopropyne 7%
'Tuzet'	mixture: thiram 40%; zinc dimethyldithiocarbamate 20%; 'urbacid' 20%
'Urbacid'	bis(dimethylthiocarbamylthio)methyl arsine

In the other 4 orchards in the Bangert area, a clean weeding system was applied. *Pratylenchus penetrans* was found in rather high numbers in sample 6.

The treatments can be classified into four groups: 1. Nematicidal treatment by DD; 2. Fungicidal treatments with captan and quintozene; 3. Broad spectrum treatments with chloropicrin, nabam and heat; 4. Apple roots. The effect of the latter treatments will only shortly be referred to and has already been discussed in 3.3.5.

Results are shown in table 17. Effects on apple soils show that broad spectrum treatments are by far the most effective. Only nabam failed to produce

TABLE 15. Some general properties of the soils used in the exploratory pot experiment. Nos. 1–6 are apple soils from the Bangert area, nos. 9 and 10 fresh soils from this area. Nos. 7 and 8 are apple soils from the South-Western, and Northern part of the country respectively.

Characteristics	Apple soils nos.								Fresh soils nos.	
	1	2	3	4	5	6	7	8	9	10
pH	7.0	7.1	7.3	6.9	7.2	7.3	6.5	4.9	7.2	7.2
Humus %	4.6	5.3	5.1	6.0	4.5	3.3	3.3	18.0	6.4	3.2
$CaCO_3$ %	3.6	5.6	6.9	2.6	3.9	2.9	0.1	0	3.4	1.2
Clay fraction <0.002 mm	16	23	20	16	20	16	21	7	21	19
Clay fraction 0.002–0.016 mm	7	14	9	9	8	8	10	13	9	7
Fine sand fraction 0.016–0.105 mm	63	45	55	60	60	66	59	25	55	66
Coarse sand fraction >0.105 mm	6	7	4	6	4	4	7	37	5	4
P_2O_5[1] 1/1,000 %	79	123	49	157	111	50	94	88	35	87
K[2] 1/1,000 %	35	48	32	55	50	27	50	49	39	32
MgO[3] 1/10,000 %	297	320	136	227	409	137	209	415	258	196

[1] Extraction in ammonium lactate- acetic acid
[2] Extraction in 0.1 N HCl
[3] In 1 N NaCl

TABLE 16. Exploratory pot experiment. Nematodes per 100 ml of soil at the beginning of the experiment.

	1	2	3	4	5	6	7	8	9	10
Pratylenchus		10	70	10		235	20	10	60	15
Paratylenchus	50	25	190	15	20	190	50		80	
Tylenchorhynchus	65	15	70	35		10	10	75	65	25
Rotylenchus/ Helicotylenchus	5		160	5	5	20		5	135	
Criconemoides	5		5		10			5	20	
Other *Tylenchidae*	210	195	210	75	40	125	175	180	245	185
Saprozoic nematodes	1995	1640	2665	1005	2045	1245	1065	2350	1865	1240

consistently good effects. Using a slightly higher dosage OOSTENBRINK and HOESTRA (1961) obtained rather good effect with nabam against specific replant disease of roses. All treatments in groups 1 and 2 were much less effective than the successful broad spectrum treatments. Within the group of apple soils from the Bangert area none of the three treatments in groups 1 and 2 improves growth to the level of the untreated fresh soils.

The effect of DD, though limited as compared to most broad spectrum treatments on all apple soils is still appreciable. This rather strong effect cannot be explained by the killing of nematodes, because there is no correlation with nematode numbers, which were low in most cases. With fresh soil no. 9 the effect of DD was negligible. This soil was fairly heavily infested with nematodes. This also suggests that the nematode groups involved were not very harmful to the apple rootstocks at the infestation levels present in the soil.

The effect of captan was of the same order as that of DD, and varied in much the same way from case to case. This suggests that the two treatments had a factor in common, which cannot be nematicidal, because in our experiments captan did not kill the parasitic nematodes in the soil. In this connection it is interesting to note that soil no. 6, the only soil heavily infested with *P. penetrans*, is also the only one in which captan shows much less effect than nabam, which killed the plant parasitic nematodes.

The other fungicide, quintozene, is interesting because it shows one aspect of the usefulness of including fresh soils in this type of experiments. With apple soils, quintozene had small, or negative effects, and the results with fresh soils demonstrate that this can be attributed to phytotoxicity.

Before conclusions can be drawn on the positive effects of a number of treatments, the role of the nitrogen effect generally known to occur after biocidal soil treatments has to be considered. (ALTMAN, 1963; CHANDRA and BOLLEN, 1961; GOOD and CARTER, 1965). This effect is due to an increased mineralization of nitrogen after the killing of organisms which are then easily attacked by survivers, including ammonifying bacteria, and newly introduced organisms. Since nitrifying organisms are highly susceptible to many chemicals used for soil disinfection, there is first a strong accumulation of ammonial nitrogen.

TABLE 17. Exploratory pot experiment 1959/1960. Length of newly formed shoots in *cm* per plant (controls) and in % of controls.

Treatments	Dosage/l of soil	Soils no.									
		1	2	3	4	5	6	7	8	9(fresh)	10(fresh)
Control		*15*	*17*	*22*	*17*	*14*	*11*	*22*	*23*	*46*	*34*
		100	100	100	100	100	100	100	100	100	100
DD	0.4 ml	213*	163**	146	166**	206**		153	239**	102	188**
Captan 50%	0.4 g	149	146	141	167**	221**	198	164	160	106	142
Quintozene 25%	0.5 g	116	79	84	76	130	130	97	106	37	61
Nabam	0.7 ml	146	112	157*	168**	197**	418**	205*	196*	153**	132
Chloropicrin	0.7 ml	429**	229**	180**	447**	322**	508**	250**	303**	117	202**
Heat, 60 °C, one hour		341**	266**	191**	278**	444**	332**	209*	291**	142*	158*
Heat, 120 °C, two hours		399**	286**	164**	318**	424**	418**	236**	347**	112	177**
NH_4NO_3	1.5 g	129	127	81	92		129	143	197*	114	137
Apple Roots, untreated	17.5 g	131	97	122	108	159*	116	111	132	100	93
Apple Roots, 60 °C, 2 hrs	17.5 g	127	110	86	97	123	142	101	93	111	83

This point has been investigated with soil no. 8, which was treated in March 1960 and sampled in June (table 18).

TABLE 18. Exploratory pot experiment. Total nitrogen, and ammonial nitrogen in soil no. 8, 10 weeks after treatment (mg/kg of dry soil).[1]

	Total N	Ammonial N
Control	12.5	1.2
DD	45.5	27.5
Captan	24.3	9.6
Quintozene	14.5	1.0
Nabam	42.1	15.5
CP	60.2	41.6
Heat (60 °C)	41.4	31.4
Heat (120 °C)	41.3	32.7
Fresh apple roots	18.6	1.3

[1] Thanks are due to Dr. D. A. van Schreven, Rijksdienst voor de IJsselmeerpolders, Kampen, for carrying out this analysis.

These figures demonstrate the nitrogen effect and the accumulation of ammonial N with several of the treatments. The existence of a nitrogen effect in this experiment is further substantiated by the positive effect of the treatments stimulating N-mineralization with the two fresh soils. This is most pronounced in the case of no. 10. This soil also shows the best effect after a treatment with ammoniumnitrate. Also the negative effect of the addition of apple roots (giving rise to a relative shortage of nitrogen during decomposition in these unfertilized series) suggests that the difference between the two cases in reaction to the various disinfectants, are based on the fact that the resources of nitrogen were smallest in soil no. 10, thus giving rise to a stronger stimulation of growth by chemicals than in soil no. 9. Although these observations relate the effect of a number of soil treatments to a nitrogen effect, it remains to be explained why the treatment with ammoniumnitrate of fresh soils does not improve growth as much as some of the chemical treatments. It is suggested that in fresh soils organisms occur, that are slightly harmful to apple and removed by some of the treatments.

With apple soils from the Bangert area, the effect of ammoniumnitrate has been very small, though by the darker colour of the foliage in the treated pots, as compared with the controls, it may be concluded that nitrogen has been taken up by the roots and transported to the leaves. Under these conditions growth of the plants is not improved. This means that the positive effect of any treatment cannot be attributed to nitrogen effect alone.

If the causal factor of SARD is removed from the soil by a treatment which also produces a nitrogen effect, the improved growth is the combined result of the control of the causal factor and the nitrogen effect. Since the nitrogen effect is a common factor in treatments improving growth, differences in growth improvement can be attributed to differences in control of the causal factor of

SARD. Thus chloropicrin and heat were the most effective in controlling SARD, and the comparison of growth in the treated apple soils and in fresh soils indicates that control was complete.

The DD effect could not be explained on the basis of nematode kill, nor on the nitrogen effect alone, thus it must be concluded that DD controlled the causal factor of SARD to some extent. This aspect of DD activity may be related to the conditions under which the experiments were carried out, and will be discussed in more detail in section 4.4.2.

The effects of nabam and captan can likewise be attributed to partial control of SARD.

All 'Bangert' apple soils perhaps with the exception of no. 3 seem to have been heavily infested with the causal factor of SARD. Soil no. 8 seems to have been rather heavily infested when CP and heat effects are considered, but the strong effect of ammoniumnitrate suggests that the disease was not so serious here, and that the effects of various chemicals may be explained to an appreciable degree by nitrogen effect.

Summarizing it can be said that the experiment showed a good reproduction of SARD and suggested general occurrence of the disease in the region where most samples had been taken, the Bangert area. The disease was controlled by broad spectrum treatments and it was evident that nematodes did not play an important role.

4.3. Field experiments

4.3.1. *Introduction*

Some small scale field experiments were established in 1959 and 1960. From 1961 onwards, each year several field experiments were started. A total of 65 field experiments were spread over all fruit growing areas (fig. 9). For each major fruit growing centre, one or more of these experiments will be discussed in this chapter. The main conclusions from other experiments will be briefly mentioned.

Special technical details will be mentioned with the experiments concerned. General data are shown in table 19, and the nematode infestations in table 20. Unless otherwise stated plots treated with chloropicrin were covered with plastic (transparant polyethylene of 0.02 mm thickness) immediately after injection. Methyl bromide was applied under plastic (0.1 mm thick polyethylene), which was placed before treatment. Plastic was left on the plots for one week in early applications (September). This period was extended to 3 or 4 weeks in the case of chloropicrin treatments at low soil temperatures.

4.3.2. *Province of North Holland*

Eight field experiments were carried out, of which 7 in the Bangert area, in which experiments 1 and 2 were located. The Bangert area is one of the oldest fruit growing areas in the Netherlands. It was known for fruit production as early as the seventeenth century. Recently the area planted to fruit was extended considerably. From 1938 to 1964 total acreage more than doubled (Rijnierse and

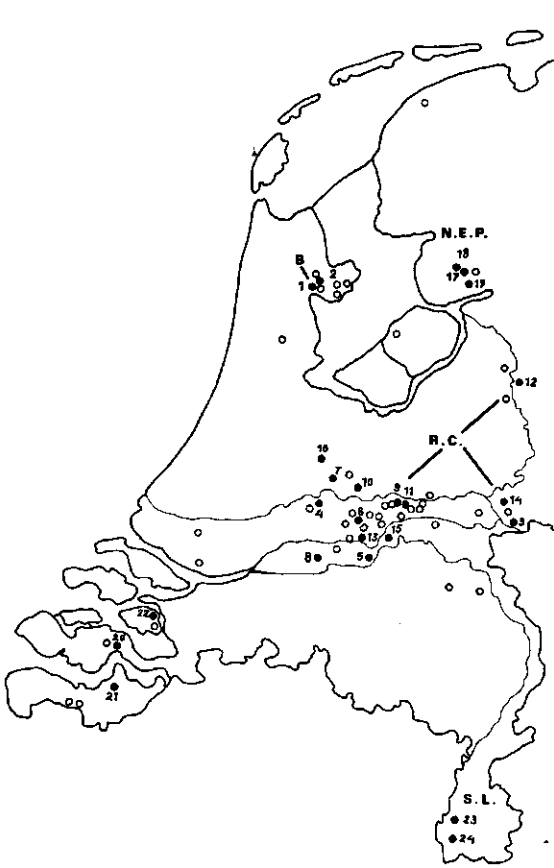


FIG. 9. Location of field experiments. Black dots: experiments described in section 4.3. B: Bangert area. S.L.: South Limburg, N.E.P.: North East Polder, R.C.: River clay area.

VAN VEEN, 1966). In some villages virtually all the land is now planted to fruit, of which 40% pears and 60% apples.

Field experiment no. 1. The purpose of this experiment was to test some treatments already applied in other areas, on a typical Bangert soil, and to demonstrate the effect of soil fumigation to growers in this area. As a special treatment a heavy dose of stable manure was included. The results are shown in table 21.

CP strongly stimulated growth of the trees. Stable manure does not improve growth at all. When comparing growth of controls with growth of trees on CP treated plots in preceding years, it can be seen that the lead of the latter on the controls is about one year. In this experiment it is also noteworthy that the effect of DD and chlorobromopropene is not much less than that of CP.

Experiment 2. The apple orchard in which this experiment was established

TABLE 19. General data on field experiments Nos. 1-25.

No.	Region	Soil pH	Number of treatments	Number of replicates	Number of trees per plot	Date of treatments	Soil temp. at 20 cm at date of treatment (°C)
1	Bangert	7.3	6	6	6	Oct. 16.62	11.0
2	Bangert	7.0	5	7	1	Oct. 11.63	10.0
3	River clay area	6.9	8	6	6	Sept. 8.61[1]	17.5[1]
4	River clay area	7.0	7	4	7	Sept. 27.61	17.0
5	River clay area	6.9	8	6	6	Sept. 27.61	18.5
6	River clay area	6.7	8	6	6	Oct. 13.61	14.0
7	River clay area	5.5	8	6	6	Sept. 19.61	18.5
8	River clay area	6.5	8	6	6	Sept. 29.61[1]	17.5[1]
9	River clay area	6.7	8	6	6	March 21.62	2.0
10	River clay area	5.6	8	6	6	Nov. 7.61	5.5
11	River clay area	6.1	8	6	6	March 2. 62	3.0
12	River clay area	7.1	8	6	6	Oct. 11.61	12.5
13	River clay area	6.7	8	6	13	Oct. 25.63[2]	11.5[2]
14	River clay area	6.7	2	4	4	Nov. 7.63	10.5
15	River clay area	7.2	6	9	1	Sept. 27.65	15.5
16	River clay area	6.9	5	4	2-3	Sept. 23.64	12.0
17	North East Polder	7.3	9	5	2	Nov. 22.63	8.0
18	North East Polder	7.4	9	4	2	Oct. 5.65	13.0
19	North East Polder	7.2	9	3	5	Oct. 5.65	13.0
20	South-West	6.3	4	5	2	Oct. 30.63	7.0
21	South-West	7.1	4	7	1	Nov. 26.63	8.0
22	South-West	7.0	7	8	1	July 30.64	19.5
23	South Limburg	6.5	8	10	1	Nov. 3.64	6.5
24	South Limburg	6.2	8	10	1	Nov. 3.64	6.5
25	East	4.7	6	6	9–11	Oct. 19.62	10.0

[1] MIT applied Oct. 27.61; soil temp. 10°C.
[2] Two additional CP series trated on Nov. 13.63 and Jan. 10.64 at soil temp. of 7 °C and 3 °C respectively.

was known to be infested, from the observation that trees on the site of a former large path-way were growing much better than trees on apple soil in the adjoining rows. There were practically no plant parasitic nematodes present. In table 22 it can be seen that DD at the 'normal' rate of 60 ml/m² (as recommended for nematicidal action) has little effect in comparison with all other treatments. A high dosage of DD, which is far more effective than the normal dose, equals methyl bromide, also applied at a rather high rate. CP was again the best treatment.

In a third experiment, with the usual strong effect of CP, the same difference between the two dosages of DD was observed.

There were also 4 experiments in which machinery was used to apply the fumigants. The first, treated on 8 October 1963 with DD, chlorobromopropene, and CP, using a fumigating machine for light greenhouse soils, yielded little result. This was caused by the superficial and irregular injection of the fumigants into the wet and rather heavy soil of this field. It thus became obvious that for

TABLE 20. Nematode infestation per 100 ml of soil at the beginning of the field experiments 1–25.

No.	*Prat.*	*P.p.*	*P.f.*	*Para*	*Tyl.*	*Rot/ Hel.*	*Crico.*	*Tricho.*	O.T.	Sapr.
1				130	195	5	75	15	260	1915
2					5			5	180	4910
3	10			95	5	5		5	145	900
4	320	65	160	85	45	15	25		60	1090
5	5			120	60	5		5	130	1760
6	90	10		50	10	5			75	715
7	185	85[1]		375	25	35			265	1910
8	65	20[1]		25	5	105			95	230
9	155	55[1]		605	30	60	30		250	1115
10	20				5	15		5	170	1355
11	80	15[1]		405	45	15			280	940
12	195	80[1]		75	15	50			150	1210
13	260		75	190	35	85			350	1130
14	85			20		260			60	775
15	205	35	70	10	25	5			95	3970
16	20			255	65		10		295	520
17	10			190	25	10			70	2615
18	5			125	5				220	2300
19	10			615	15				255	2350
20				150	5				350	1220
21	85	10		120	70				220	1480
22	20			10	25	5			140	905
23	40			310	35	275	10		190	2250
24	75			15	15	20			170	2065
25	85	30	30	80	600				210	2600

Prat.–Pratylenchus, P.p.–Pratylenchus penetrans, P.f.–Pratylenchus fallax, Para.–Paratylenchus, Tyl.–Tylenchorhynchus, Rot/Hel.–Rotylenchus and *Helicotylenchus, Crico.–Criconemoides, Tricho.– Trichodorus,* O.T.-other *Tylenchidae,* Sapr.–Saprozoic nematodes.

[1] These populations of *P. penetrans* may have been mixed populations of *P. penetrans* and *P. fallax*

the application of fumigants on heavy soils under unfavourable conditions, special machinery was needed. In 1964, such a machine was available (fig. 14), with which a strip, 1.30 m wide, can be treated at one time. A plastic sheet has to be laid by a separate machine which follows the first at a close distance.

In this way three experiments were established of which the results were mostly comparable to those of the experiments treated with the hand injector. CP had a very good effect and was the best treatment; CBP was better than DD but clearly less effective than CP, while DD (standard dose) had little effect. These results were the same in all three cases, though in only one case apple was planted after apple. In one of the two others pear was planted after a mixed apple-pear plantation, and in the third apple after pear.

4.3.3. *The river clay area*

The interest in this area does not only result from its importance for fruit

TABLE 21. Results of field experiment 1, Variety: Cox's Orange Pippin/M. II.

Treatment	Dosage (per m²)	Shoot growth (*dm* for controls, % of controls for all treatments)[1] Year			Production (kg) Year	
		1st	2nd	3rd	2nd	3rd
Control		*15*	*59*	*214*	0.98	2.2
		100	100	100		
DD	60 ml	267**	248**	170**	1.44	3.0
CP	40 ml	273**	299**	201**	1.05	3.3
Stable manure	40 kg	100	112	100	0.98	1.7
CP + Stable manure	40 ml + 40 kg	260**	290**	218**	1.22	3.3
CBP	60 ml	287**	246**	155**	2.07	4.0*

[1] In other tables on field experiments the same system will be followed, briefly indicated as (*dm* and %).

TABLE 22. Results of field experiment 2. Variety: Golden Delicious/M. VII.

Treatment	Dosage (per m²)	Shoot growth (*dm* and %) Year		Production (kg) Year		
		1st	2nd	2nd	3rd	4th
Control		*29*	*134*	0.9	2.4	7.5
		100	100			
DD	60 ml	113	106	1.6	4.3	8.9
DD	140 ml	159	139	2.3	6.1	16.8**
CP	50 ml	241**	166	2.3	6.7**	10.8
Methyl bromide	100 g	155	131	1.1	4.7	14.8**

growing and the extensive need for replanting, but also from the fact that the degree of the infestation with SARD varies more within this area than in the other fruit growing centres.

Experiments 3–12. This series of ten field experiments was established in autumn 1961 and spring 1962. Of these trials, 9 were on apple soil, and one on fresh land (no. 12). Some of the results over the first three growing seasons have already been published (HOESTRA et al., 1964). In this section, part of this work will again be reviewed, together with later results (tables 23-25).

In the first year (table 23), growth on the control plots of the experiment on fresh soil, no. 12, was better than growth on almost all the untreated apple soils. The effect of most of the treatments on fresh soil was slight, as is also shown by the figures in table 25.

TABLE 23. Field experiments 3–12. Shoot growth in first year (*dm* and %) Variety: James Grieve/M. IV.

Treatment	Dosage (per m²)	Experiment number									
		3	4	5	6	7	8	9	10	11	12
Control		*2*	*6*	*7*	*4*	*14*	*10*	*5*	*3*	*6*	*14*
		100	100	100	100	100	100	100	100	100	100
DD	60 ml	170	172	125	314	88	184	138	115	113	127
CP	40 ml	325**	453*	250**	569**	237**	305**	81	219*	113	99
CP	80 ml	605**	496*	293**	1053**	214*	336**	198**	265**	138	128
CBP	60 ml			168**	606**	139					162**
MIT	150 ml	135					142	98	162	85	
Metam	100 ml	175	156	109	131	154	150	67	165	93	103
Steam[1]			765**								
Captan	100 g	150	133	96	133	119	180	75	135	72	105
Captan[2]		120		81	75	88	151	88	119	95	134

[1] 90°C during one hour.
[2] root dipping treatment in slurry.

TABLE 24. Field experiments 4,5,6 and 8. Shoot growth in the second and third year after planting (*dm* and %). Dosages: see table 23.

Treatment	Number of experiment, year							
	4		5		6		8	
	2nd	3rd	2nd	3rd	2nd	3rd	2nd	3rd
Control	*68*	*136*	*39*	*54*	*50*	*85*	*84*	*144*
	100	100	100	100	100	100	100	100
DD	147	111	133	120	176**	150**	117	118
CP (40 ml)	199**	125	186**	170**	235**	181**	166**	139
CP (80 ml)	208**	130	196**	204**	316**	224**	178**	153
CBP			127	136	204**	136**		
MIT							117	109
Metam	136	110	132	129	124	114	119	113
Steam	311**	189**						
Captan	101	102	101	106	106	97	126	131
Captan (root dip)			110	109	94	90	111	118

TABLE 25. Field experiments 3,4,5,6,8 and 12. Production in kg per tree. Dosages: see table 23.

Treatment	Number of experiment, year.							
	3	4	5	6			8	12
	3rd	3rd	3rd	3rd	4th	5th	3rd	3rd
Control	0.2	2.0	0.5	2.2	5.1	4.7	2.8	2.6
DD	0.7	2.8	1.1	3.9*	6.6	6.5	3.4	2.6
CP (40 ml)	1.7**	4.6**	1.3	5.5**	9.2**	7.5	4.3	1.8
CP (80 ml)	2.6**	4.6**	1.9**	6.4**	11.3**	8.0*	5.4**	2.7
CBP			0.7	4.3**	8.2**	7.6		2.7
MIT	0.6						3.4	
Metam	0.5	3.2	0.6	2.6	5.1	4.8	3.8	2.3
Steam		6.1**						
Captan	0.4	2.6	0.8	2.9	6.2	5.6	3.5	2.1
Captan (dip)	0.1		0.9	2.4	5.0	5.2	3.2	2.5

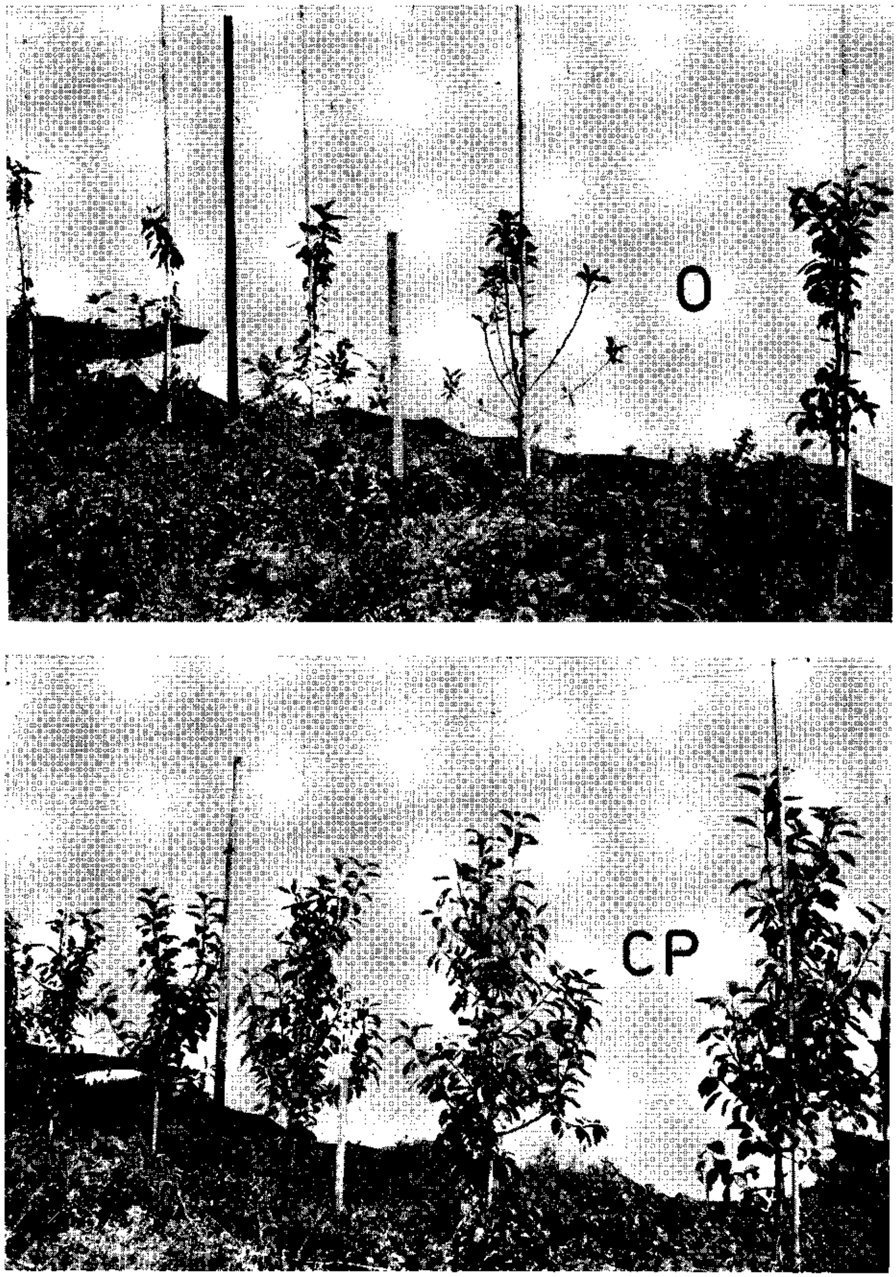


FIG. 10. Field experiment 6. Growth of apple, James Grieve/M. IV, on untreated (O) and CP treated soil. Pictures taken at the end of the first growing season.

The two fields treated in spring (9 and 11) showed symptoms of phytotoxicity on the chloropicrin treated plots. From the results on all other apple soils, the favourable effect of CP treatment is very clear (figure 10). Heat, soil kept at 90 °C for one hour, applied in one case, appears to be even more effective. CBP is the second best of the soil fumigants. All the other treatments were not very effective, as compared with CP, but in most cases positive effects were measured, for instance with DD and metam. Although the differences with the controls were not found significant for these products when individual fields are considered, the fact that in almost all cases growth on treated plots was better than on untreated ones, is in itself a significant observation.

The recovery of trees on untreated soil is clearly illustrated by this series of experiments; growth in the second year is better than on CP treated soil in the first year. In the third year, however, shoot growth of control trees is somewhat inferior to growth on the CP treated plots in the second year.

Field experiment 13. In 1963 a field experiment was established mainly to test the effect of application dates of chloropicrin. Some other fumigants were included, and an organic amendment, peat. In 1963 wheat had been grown. The field had been grown with apple during 40 years, until 1961. Wheat volunteer plants appeared in 1964, and were the main weed in the first part of the season. No vegetation appeared on the CP and methyl bromide treated plots. On DD and CBP plots growth of wheat was moderately reduced as compared with the control. Early in the growing season of 1964, symptoms of phytotoxicity appeared on all CP treated plots; leaves remained small and folded, and there was little new growth until late summer. As the figures show (table 26) the effect of CP was small, as compared with what has been observed in many other field experiments, and there was little difference between the application

TABLE 26. Field experiment 13. Shoot growth in first year after planting (*dm* and %). Varieties: Cox's Orange Pippin and Golden Delicious, both on M. IX.

Treatment	Dosage	Variety	
		Cox's	Golden Delicious
Control		*7.1*	*9.5*
		100	100
CP[1]	50 ml/m²	114	115
CP[2]	50 ml/m²	119	108
CP[3]	50 ml/m²	108	88
Methyl bromide	100 g/m²	211**	207**
CBP	60 ml/m²	120	130
MIT+DD	60+30 ml/m²	126	125
Peat	8 l/tree	96	96

[1] Applied 25 Oct. 1963, together with the other fumigants.
[2] Applied 13 Nov. 1963.
[3] Applied 10 Jan. 1964.

dates. The strong effect by methyl bromide also demonstrates that phytotoxicity on the CP treated plots was serious, and that the risk of phytotoxicity in methyl bromide application is smaller than in CP application. This can be related to the difference in boiling point of the two fumigants (methyl bromide: 4 °C, chloropicrin:112 °C). The treatments with CBP and with the combination of DD and methyl isothiocyanate did not result in phytotoxicity symptoms. This indicates that the plastic cover applied on CP plots, was one factor in creating conditions for the occurrence of phytotoxicity, though the plastic sheets were not removed on the same date. The weather during late winter and early spring was wet, and this has probably prevented the escape of CP residues after the removal of the plastic tarpaulins. Soil structure was rather poor in this case, and, in the rainy season, a slicky layer is formed on the surface, especially on unprotected soil as in the case of the CP treated plots. In the following year a small scale experiment was carried out in which attempts were made to reproduce the phytotoxicity symptoms in a late application of CP (15 December 1964, soil temp. 7.5 °C). The following year, the test trees did not show any signs of phytotoxicity. It should also be noted, that in the year before, other field experiments were established in the same season as the first years treatments of experiment no. 13, but that the former (nos. 2, 14, 17, 20, 21) did not show any phytotoxicity by chloropicrin on the trees planted in spring 1964. This shows that the occurrence of phytotoxicity is due to the interaction of different factors, of which the wheather conditions and the soil condition no doubt play the most important roles.

The addition of peat did not result in a better growth. There was, however, a better stand of the trees during the first six weeks after planting, but at the end of the season this effect was no longer noticeable.

Experiment 14. This small scale experiment is mentioned because it shows the use of a pilot experiment in an existing orchard as a basis for a decision on the need to treat the soil after grubbing of the orchard in the following year. Four plots were cleared in the existing orchard. Half of each plot was treated with chloropicrin, the other half was left untreated.

The effect was very strong for both varieties planted and continued through-

TABLE 27. Field experiment 14. Average growth per tree in the first two years, increment of stem circumference, and shoot growth. Varieties: James Grieve/M. I and Schone van Boskoop/M. VII.

		James Grieve				Schone van Boskoop			
		Stem increment (mm)		Shoot growth (dm)		Stem increment (mm)		Shoot growth (dm)	
Treatment	Dosage (ml/m²)	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr
Control		2	21	18	55	3	11	11	21
CP	60	11**	30**	41**	114**	5*	18	18*	51**

out the second season (table 27). It is interesting to note that both rootstocks used induce vigorous growth of the scion under normal conditions. This shows that SARD clearly does not only affect dwarfing rootstocks.

TABLE 28. Field experiment 15. Shoot growth in the first two years, production in the third year. Variety: James Grieve/M. IV.

Treatment	Dosage (per m²)	Shoot growth (*dm* and %)		Production (kg) 3rd year
		1st year	2nd year	
Control		*8*	*45*	1.7
		100	100	
DD	60 ml	160	119	2.9*
CP	100 ml	470**	261**	5.7**
Methyl bromide	100 g	370**	203**	4.1**
Telone PBC	60 ml	345**	179**	3.7**
Sulphur	600 g	58	84	1.6

Experiment 15. In this experiment (table 28, figure 11) single tree plots were used. The dosage of CP was very high due to an error. The other part of the orchard was treated entirely with CP at the normal rate of 50 ml/m². Results indicate that the high dosage was not more effective than the normal dosage applied to the other part of the orchard by commercial machinery. Methyl bromide and telone PBC also had a strong effect. With the latter product – a mixture – the dichloropropene component is not likely to be the cause of the observed strong effect, unless there is synergism. It seems more probable, however, that chloropicrin and/or 3-bromopropyne, both present in small quantities, are responsible for the favourable effect of the product. Sulphur had no effect, probably because the initial pH of 7.1 was reduced only to 6.7.

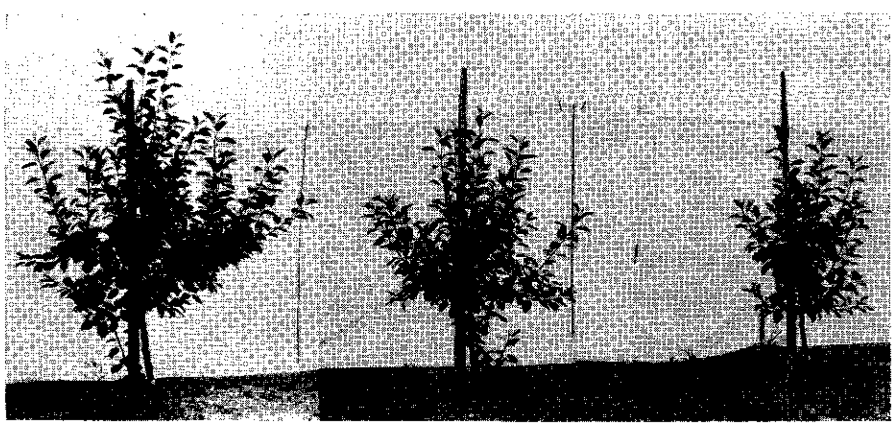


FIG. 11. Field experiment 15. Picture taken in the middle of the third growing season. Variety: James Grieve/M. IV. From left to right: CP, DD, and control.

Experiment 16. This trial was designed to compare the effect of controlling SARD on growth of apple and pear. The field is primarily a pear variety trial. Planting distances with two of the three pear varieties to be tested were such that apples could be interplanted and left for the first three years. The results are shown in table 29. One pear variety did not grow well, because of incompatibility of scion and rootstock, and is not further mentioned. The figures show that both apple and pear reacted in about the same way to the treatments, though, especially in the second year, the effects with apple were strongest. CP and methyl bromide were again the best treatments. There was an indication that a double plastic sheet over the plots increased the effect of CP. On an average, the effects of DD, chlorobromopropene, metam, and formalin were moderate as compared to the CP effects.

TABLE 29. Field experiment 16. Shootgrowth in the first two years. Pear varieties: Bonne Louise d'Avranches/Quince A and Précoce de Trévoux/Quince A. Apple: Golden Delicious/M. IX.

Treatment	Dosage (per m²)	Bonne Louise (*dm* and %)		Précoce (*dm* and %)		Golden Delicious (*dm* and %)	
		1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr
Control		*27*	*89*	*16*	*62*	*23*	*63*
		100	100	100	100	100	100
DD	60 ml	142	123			121	147
CP	50 ml	145	142*			153*	164*
CP[1]	50 ml			189	147*	193*	204**
CBP	60 ml	132	111			128	130
Methyl bromide	100 g			171	126	158*	149
Metam	100 ml			161	118	124	130
Formalin	600 ml			152	119	115	127

[1] Covered with a double layer of polyethylene 0.02 mm.

The difference in reaction between apple and pear to CP treatment was more pronounced in a small trial on the same farm, on soil heavily infested with SARD. Quince rootstocks made reasonable growth on untreated plots, where growth of apple rootstocks M. IV was almost nil.

Other experiments in the river clay area included a trial with a range of apple rootstock/scion combinations on apple soil severely infested with SARD. Different soil treatments were applied, namely CP, metam as well as several dosages of peat, compost and mixtures of the two, worked into the soil at the planting site. Effect of CP was very strong in most varieties, although the number of trees was not sufficient to make reliable comparisons between varieties. Metam and all the treatments with organic materials were ineffective.

Another trial served to compare different ways of applying metam, which is normally injected, but in this experiment was also washed into the soil, using large quantities of water. With both application methods the compound proved ineffective.

In an experiment CP treated commercially with machinery, production of the controls in the third year was about 2.2 kg per tree for Golden Delicious on rootstocks M. II and 26 and on MM. 111. Production of CP treated trees was about 5.5 kg for all three scion/rootstock combinations (ANONYMOUS, 1968a). Shoot growth was affected in about the same way in the first two years. Peat had little effect on either CP treated or untreated plots.

4.3.4. *North East Polder*

The North East Polder, in the former Zuiderzee, was reclaimed in 1941. Some areas were reserved for fruit growing, and the first orchards were planted in 1945, mostly with apples.

Apple replant disease was quite soon observed. Growers often used part of their land first as a nursery, to raise the trees for the young plantations on their own farm. In quite a number of cases it was reported, that poor growth of the young orchard occurred on the sites of the former nurseries. The growth differences can still be seen at present.

With these experiences in mind, it seemed interesting to start some field work in this region, when, from about 1965, growers started to renew some of the then 20-year-old plantations.

A preliminary experiment showed little effect of DD, and a rather strong effect of CP. Half of the replicates were treated with leaves of sugar beets at the rate of 90 tons per ha. This treatment was supposed by some growers to improve the growth of replanted apples. In the experiment there proved to be no difference between the plots treated and not treated with sugar beet leaves.

Experiment 17. This trial was established on the former site of a ten-year-old orchard. The two formulations of tridipam (MIT group of compounds) were reported by the manufacturer to have fungicidal as well as nematicidal effects. In another treatment fresh asparagus roots were worked into the soil because asparagus was mentioned as particularly favourable when preceding apple under field conditions (SCHANDER, 1956). The figures (table 30) show that CP was the only effective treatment. DD, at the increased dosage of 120 ml/m^2 was clearly phytotoxic.

Experiment 18. At the same farm as no. 17, another field experiment was started, of which the results are shown in table 31. Chloropicrin, methyl bromide, mixtures containing 3-bromopropyne and one of the two first mentioned compounds, or both, were very effective. DD holds an intermediate position. Sulphur had no effect on growth so far. The pH was 7.4 originally, and was lowered to 7.3 and 7.2 by the low and high dosage of sulphur respectively.

Experiment 19. This trial was established simultaneously with no. 18, in another village, on the former site of an apple nursery of an experimental fruit farm. The treatments were essentially the same (table 32).

TABLE 30. Field experiment 17. Results of the first three years. Variety: Golden Delicious/M. IX.

Treatments	Dosage	Shoot growth (*dm* and %)		
		1st year	2nd year	3rd year
Control		*15*	*54*	*128*
		100	100	100
DD	60 ml/m²	100	105	103
DD	120 ml/m²	51**	77	97
CP	60 ml/m²	252**	197**	154**
CBP	60 ml/m²	82	98	110
Tridipam 90%	24 g/m²	123	117	124
Tridipam 50%	44 g/m²	107	109	109
Peat[1]	20 l/tree	86	87	90
Fresh asparagus roots	3 kg/tree	93	94	93

[1] mixed into the soil at time of planting.

TABLE 31. Field experiment 18. Results of the first two years. Variety: Golden Delicious/M. IX.

Treatment	Dosage (per m²)	Shoot growth (*dm* and %)	
		1st year	2nd year
Control		*14*	*32*
		100	100
DD	60 ml	171*	195*
CP (plastic cover)	50 ml	236**	227*
CP (water seal)	50 ml	236**	201*
Methyl bromide	100 g	271**	220*
Trizone	30 ml	257**	250**
Telone PBC	60 ml	221**	216*
Sulphur	500 g	121	127
Sulphur	1250 g	79	92

TABLE 32. Field experiment 19. Results of the first two years. Variety: Golden Delicious/M. IX.

Treatment	Dosage (per m²)	Shoot growth (*dm* and %)	
		1st year	2nd year
Control		*19*	*89*
		100	100
DD	60 ml	116	106
CP (plastic cover)	50 ml	189**	145*
CP (water seal)	50 ml	216**	142*
Methyl bromide	100 g	216**	166**
Trizone	25 ml	142	112
Telone PBC	60 ml	153*	122
Sulphur	500 g	105	112
Sulphur	1000 g	89	91

The effects of the mixed fumigants and of DD were less than in the case of no. 17. The application of a water seal was at least as effective as the plastic cover in the case of the CP plots.

4.3.5. *South-Western part of the Netherlands*

This area is important for growing fruit, and is characterized by being situated in the 'delta' region consisting of islands and peninsulas (now mostly connected by bridges and dykes), and coastal areas. The soil is generally sea clay. Most of the field experiments in this area had single tree plots.

Experiment 20. Established on the site of a former 40-year-old apple orchard grubbed 4 years before. The results are shown in table 33. CP was the best treatment. There also seemed to be some effect of mixing peat with the soil at the planting site.

TABLE 33. Field experiment 20. Results of the first year after planting. The experiment consisted of two sections. Variety: Stark's Earliest/M.IX.

Section	Treatment	Dosage	Shoot growth (*dm* and %)
I	Control		*14*
			100
	Ditrapex	75 ml/m²	157
	Chloropicrin	70 ml/m²	300**
	Peat	10 l/tree	150
II	Control		*18*
			100
	CBP	70 ml/m²	122
	Chloropicrin	70 ml/m²	200**
	Peat	10 l/tree	117

TABLE 34. Field experiment 21. Results of the first year after planting. Variety: Cox's Orange Pippin/M. IX. (Three year old when planted).

Treatment	Dosage (per m²)	Shoot growth (*dm* and %)
Control		*27*
		100
Chloropicrin	70 ml	228**
Methyl bromide	100 g	201**
Ditrapex	70 ml	104

Experiment 21. In this trial (former orchard 17 years old) three-year-old planting material was used; the figures show that also with these older trees, strong effects were observed with chloropicrin and methyl bromide (table 34).

Experiment 22. This experiment, with treatments made in summer, was established on the former site of a 20-year-old apple orchard. The results (table 35) show the same good effects of CP treatment as usual. There was some effect

TABLE 35. Field experiment 22. Results of the first year after planting. Variety: Benoni/M. IX.

Treatment	Dosage (per m²)	Shoot growth (*dm* and %)
Control		*16*
		100
DD	30 ml	96
DD	60 ml	80
CP	30 ml	154*
CP	60 ml	187**
CP+DD	30+30 ml	163*
CBP	60 ml	92

of the dosages applied, but 30 ml/m² was already rather effective.

The results of six other experiments were generally the same, showing strong effects by CP and methyl bromide, and moderate to slight effects by methyl isothiocyanate, DD, and chlorobromopropene. Thus, in the south west, the results are generally comparable to those from experiments in other areas already discussed.

WERTHEIM and TOORENAAR (1968) reported a strong response to CP treatment in the case of apples, James Grieve/M.IX, and no effect on growth of pear, Conference/quince A, on the site of a former, 12 year old apple orchard in this area.

4.3.6. *South Limburg*

This region is interesting because fruit growing is practised on loess soils, of generally fairly low pH. The impression among growers and extension officers is that SARD is not very important in this area. This was also suggested by the results of a series of pot tests with samples from South Limburg, including samples from the two fields chosen for the experiments. It was thought interesting to see whether field experiments would yield the same results. The results of the two experiments (nos. 23 and 24) are shown in table 36. On the whole, the effects were only slight, and the effects of the fumigants were such as would

TABLE 36. Experiments 23 and 24. Results of the first two years. Varieties: no. 23: Cox's Orange Pippin/M.IX; no. 24: Golden Delicious/M.IX.

Treatment	Dosage	Shoot growth (*dm* and %)			
		Exp. no. 23		Exp. no. 24	
		1st year	2nd year	1st year	2nd year
Control		*26*	*110*	*27*	89
		100	100	100	100
DD	60 ml/m²	131	127	119	118
CP	50 ml/m²	138	137	133	125
Methyl bromide	100 g/m²	142	126	137	126
CBP	60 ml/m²	119	103	126	114
Stable manure	10 kg/m²	125	114	115	110
Compost	5 kg/m²	108	100	91	100
Peat	5 l/tree	119	107	122	124

be expected on fresh soil. In these experiments also, the impression is that the effects of fumigants, especially CP and methyl bromide, are stronger than the treatments with organic amendments.

4.3.7. *Eastern part of the Netherlands*

Experiment 25. In the north east (province of Drente) an experiment was established in a former 16-year-old orchard on sandy soil. The field had served to study the effect of adding compost to the soil. In the new experiment, only rows which had been treated with compost were used. There were treatments with DD and CP, and in addition every other tree in the experiment was treated with peat. The fumigation was realized with a machine, normally used for treating greenhouse soils. The field was infested with *P. penetrans*. In roots of control trees, 5–6,000 individuals per 10 g of fresh weight were found. The results (table 37) show that in this case, though CP is still the best treatment, DD has

TABLE 37. Field experiment 25. Shoot growth in the first two years, production in second and fourth year after planting (average per tree) Variety: Cox's Orange Pippin/M.XI

Treatment	Dosage	Shoot growth (*dm* and %)		Production (kg)	
		1st year	2nd year	2nd year	4th year
Control		*42*	*64*	0.8	6.1
		100	100		
Peat	40 l/tree	126	122	0.5	4.7
DD	60 ml/m²	121	200**	0.8	6.7
DD+peat		150**	236**	0.9	6.4
CP	40 ml/m²	131	280**	1.4**	10.8**
CP+peat		164**	295**	1.4**	9.9*

had good effects, which suggests that *P. penetrans* was the main cause of the poor growth on control plots in this case. The effect of mixing peat into the soil was again limited.

Another experiment, on sandy soil, in the south east, is noteworthy because spring application of CP resulted in phytotoxicity, on plowed land, but not on other plots in which the soil was better loosened by deep mixing to 70 cm with a 'cerejac' machine. Methyl bromide was not phytotoxic on plots with either soil tillage.

4.4. GREENHOUSE POT EXPERIMENTS

4.4.1. *Introduction*

The data on the effects of single treatments or groups of more or less related treatments, are presented in separate sections. The average length of the seedlings in the control series is indicated, and sometimes also of other treatments. In all cases the effects of the treatments are given in % of controls. Use of 'M' soil is indicated in the tables (cf. section 3.3.1.).

4.4.2. *Dichloropropene-dichloropropane mixture*

The analysis of numerous soil- and root samples confirmed that DD is a reliable nematicide. In all experiments it killed all, or nearly all, plant parasitic nematodes. In the foregoing sections it has already been shown that in most cases the effect of the DD treatment on growth of the plants on apple soil is small as compared with the effect of broad spectrum treatments. This is a valuable indication that the soils used in the experiments are infested with SARD, and therefore DD was included as a standard treatment in the pot experiments. When soil samples from one orchard were used for various experiments, it was not found necessary to include a DD treated series in all these experiments.

In one experiment the effect of DD was analysed somewhat further. Different dosages of DD were applied. The results are shown in table 38.

TABLE 38. Effect of different dosages of DD on growth of apple seedlings on 'M' soil.

Treatment	O	DD	DD	DD	DD	DD	DD	CP
Dosage (ml/l of soil)		0.1	0.2	0.3	0.4	0.5	0.6	0.33
Length (cm/plant)	7.3	9.1	9.0	10.1	13.7*	14.5**	14.4**	15.0**
(%)	100	125	123	138	188	199	197	205

There was a light infestation with plant parasitic nematodes which were killed by all the treatments, including the lowest dosage of DD. From the results of this experiment two conclusions can be drawn: 1. The small effect of the lower dosages of DD is not due to phytotoxicity, because the higher dosages would then have given still poorer results, and 2. The higher dosages of DD are rather effective in controlling SARD. In three other cases low and high dosages were compared, with similar results. In one case very good growth occurred after the application of 1.2 ml DD per l of soil.

These results may also explain the rather strong effect observed with some of the soils used in the pot experiment discussed in section 4.2. Here the dosage was 0.4 ml/l which is twice the normal one. In the field, results of the DD treatment at the normal dosage are variable, with little or no correlation with nematode figures. This is probably the result of the widely different conditions under which the fumigant acts in the soil. If, for instance, after heavy rainfall, the soil surface is more or less sealed, the product may remain in the soil for a long time. It is known that the concentration time product is an important factor in the action of soil fumigants (HAGUE and SOOD, 1963). DD is known to affect organisms other than nematodes markedly when applied in high dosages (WENSLEY, 1953) as was confirmed in the above mentioned pot experiments, and the same effect may be expected to occur in conditions of prolonged exposure to normal dosages under certain conditions in the field.

4.4.3. *Chloropicrin*

Chloropicrin is a very effective fungicide (DOMSCH, 1959a) and general soil sterilant (REBER, 1967 a,b). Its effectivity in controlling SARD was mentioned by HOCHAPFEL (1955); ANONYMOUS (1962 a, 1966 a, b, c, 1968 b); PITCHER et al. (1966); HOESTRA and KLEIJBURG (1960); HOESTRA and VAN MARLE (1962); HOESTRA et al. (1964), and others. The compound also controls many other soil borne-diseases, including specific replant disease of cherry (PITCHER et al., 1966; ANONYMOUS, 1967 a); cf. table 4, page 23.

In the pot experiments CP (sometimes heat) was included as the standard treatment for comparison with the effect of other treatments. Thus in the following sections many examples of the CP effect will be shown in the discussion of other groups of treatments.

The dosage applied in most cases was 0.2 ml per l of soil which approximately corresponds with 50 ml per m² in the field, as recommended for practical control of SARD. A few experiments have been carried out to test the effect of different dosages (table 39).

TABLE 39. Effect of two dosages of chloropicrin with four different apple soils from the river clay area. (*cm* and %). Soil no. 3 is 'M' soil.

Treatment	Dosage (ml per l of soil)	Soil no. 1	2	3	4
Control		*10.3*	*8.3*	*8.7*	*15.9*
		100	100	100	100
DD	0.2	104	119	72	113
CP	0.1	163*	200**	225**	122
CP	0.2	137	206**	226**	121

The figures show that there is practically no difference between the normal dosage and half the normal dosage (0.2 and 0.1 ml/l of soil respectively). This illustrates the efficiency of CP in controlling SARD. In the field differences in the effect of the two dosages 40 and 80 ml/m² were quite outspoken in some cases (section 4.3.3.). This shows that it is not always easy to compare dosages in pot and field experiments, which is the result of the variable, and often unfavourable conditions in field application.

4.4.4. *Methyl isothiocyanate*

Methyl isothiocyanate (MIT) is the active principle of a range of soil disinfectants. It is either applied as such (dissolved in an organic solvent), or mixed with other soil fumigants. The effect of other products, notably metam and dazomet is also based on the activity of MIT, because MIT is released with the breakdown of these products in the soil (PEACHEY and CHAPMAN, 1966).

TABLE 40. Effect of MIT and related products, and mixtures containing MIT, on growth of apple seedlings on apple soils. (*cm* and %)

Treatment	Dosage (per l of soil)	Origin of samples					
		Bangert		South-West	'M' soil		
Control		*2.8*	*6.8*	*4.0*	*4.5*	*5.7*	*7.3*
		100	100	100	100	100	100
DD	0.1 ml	121	99	128		111[1]	123[1])
CP	0.2 ml	257**	175**	275**	454**	228**	205[2])**
MIT	0.5 ml					111	
Ditrapex	0.2 ml				207*	115	
	0.3 ml					116	
Dazomet	0.2 g	121	134	183*		110	
	0.3 g					128	
Metam	0.4 ml						147
EP 201	0.2 ml					207**	
	0.3 ml					207**	

[1]) 0.2 ml/l of soil.
[2]) 0.33 ml/l of soil.

Table 40 shows the result of 6 pot experiments in which one or more of these products were tested. The figures show the same tendency as the field experiments; the effect is small or moderate (cf. SAVORY, 1967). The only exception is EP 201, which had a strong effect, probably because of the small quantity of CP present in this mixture.

It was found interesting to consider the poor effect of MIT from the same point of view as has been done in the case of DD, namely, with regard to the elimination of possible causal factors of SARD (HOESTRA, 1967). MIT effectively controlled plant parasitic nematodes in our experiments. In the field, a fairly strong weed killing effect was observed. The products are also known as good soil fungicides (BOLLEN, 1961; DOMSCH, 1963 b; GOOD and RANKIN, 1964; REBER, 1967 a, b). The latter aspect seemed extremely interesting and therefore some experiments were done on the survival of micro-organisms after a treatment with MIT of apple soils strongly infested with SARD on which poor growth was later observed. A dilution plate method was applied using two media: potato dextrose agar with 50 ppm terramycin, and soil extract agar. The dilution method and the soil extract agar are described by POCHON and TARDIEUX (1962). In one experiment there were 19.10^4 fungal propagules per g of untreated soil, and only 6.10^2 after treatment with MIT. Numbers of bacteria and actinomycetes were not reduced by the fumigant; numbers found were 90.10^5 and 97.10^5 per g of untreated and MIT treated soil respectively. In other experiments, the failure of MIT to control bacteria and actinomycetes was confirmed (cf. REBER, 1967 a, b). It is evident that these results suggest that fungi are not involved in SARD as essential factors. Further work on the effect of MIT on soil microflora of apple orchards is needed before definite conclusions can be drawn.

4.4.5. *Other fumigants*

Data on the effect of other fumigants tested are shown in table 41. The nematicidal action of all these products was good. As in the field, chlorobromopropene and formalin have little to moderate effects on growth. Both products are known as moderately effective general soil sterilants (WELVAERT and VELDEMAN, 1959). Ethylene dibromide is quite a specific nematicide (WENSLEY, 1953) and it does not improve growth. Only propylene oxide, which is a good general soil sterilizer with little side effects on general soil fertility (ALDRICH and MARTIN, 1952; ARK, 1947; LIESE and AMMER, 1965), equals the effect of chloropicrin.

4.4.6. *Various chemicals*

Into this group products are brought together which have little or no fumigant action. Several of these chemicals are applied to control soil-borne fungal diseases. Some are not phytotoxic, at least at certain dosages, and have therefore been called 'cultural fungicides' (WELVAERT, 1962) to indicate that application nearby or on living plants is possible. In the experiments, of which the results are shown in table 42, the treatments were carried out before planting. Temik is the only specific nematicide in this group. Its nematicidal action was good; there was no improvement in growth. The highest dosage was somewhat phytotoxic. The nematicidal action of the other products was weak in most cases. Moreover, the infestation rate of the soil with plant parasitic nematodes in all soils used was low.

Aretan is an organic mercury compound, which has strong general sterilizing effects, (DOMSCH, 1963b) and its application led to an appreciable growth improvement. S 57, an experimental mixture of fungicides including an organic mercury compound, had a fairly strong effect. In a small scale field experiment it was also reported to improve growth of apple on apple soil very markedly (VAN HELVOORT, personal communication).

The group of nitrobenzenes showed moderate to fairly good effects, but evidence of phytotoxicity in two cases, quintozene and tecnazene having reduced effects with higher dosages.

As in the field experiments, and the pot trial described in section 4.2., captan had a low to moderate effect.

The effect of the organic arsenic compounds, urbacid and rhizoctol were slight or nil. The mixture, tuzet, was somewhat better, most probably as a result of the presence of thiram, which is one of its constituents. Similar conclusions can be drawn when the effects of benquinox/thiram and benquinox are considered. Thiram was very effective in the one experiment in which the pure compound was applied. Thiram is known to reduce numbers of fungi, bacteria, and actinomycetes strongly when applied to soil (DOMSCH, 1963 b).

Not mentioned in the table are some experiments done with dexon and allylidene diacetate. Dexon was somewhat phytotoxic to apple, but nevertheless the impression was gained that it did not control the causal factor of SARD. Allylidene diacetate had no effect on growth. Both products have been reported

TABLE 41. Effect of various fumigants on growth of apple seedlings on apple soils. (*cm* and %) Soils 1–6 are from river clay area, (1, 2 and 6: 'M' soil) 7 and 8 are sea clay soils from the South-Western part of the country.

Treatment	Dosage (per l of soil)	1	2	3	4	5	6	7	8
Controls		*4.5*	*6.5*	*6.6*	*3.7*	*3.7*	*6.5*	*3.2*	*3.8*
		100	100	100	100	100	100	100	100
DD	0.2 ml		111	109	103	108	135	116	89
Chloropicrin	0.2 ml	454**	266**	156*	184**	154**	169*	200**	339**
Chlorobromopropene	0.2 ml	276**	151	167*	111	119			
Mixture of 84% dichloropropene and 16% chlorobromopropene	0.18 ml	169							
Formalin 38%	2.0 ml			114	159	116		153**	255**
Ethylene dibromide	0.13 ml						98		
	0.26 ml						111		
Propylene oxide	0.5 ml						168*		
	1.0 ml						206**		
	2.0 ml						220**		

TABLE 42. Effect of various chemicals on growth of apple seedlings on apple soils (*cm* and %). Nos. 1–6 are from field experiment no 6 ('M' soil); 7 and 8 are other river clay area soils; 9 and 10 are from the South-West; 11 is from Bangert area.

Treatment	Dosage (per 1 of soil)	1	2	3	4	5	6	7	8	9	10	11
Control		*4.4*	*5.2*	*4.6*	*5.5*	*6.8*	*6.9*	*7.3*	*11.3*	*3.8*	*3.2*	*12.0*
		100	100	100	100	100	100	100	100	100	100	100
DD	0.2 ml				111			116	163**	89	116	88
CP	0.2 ml	277**	215**		231**		209**	227**	219**	339**	200**	165**
Heat (steam-sterilized, 2 × 1 hour)						237**						
Temik 10%	0.04 g			87								
	0.2 g			91								
	0.4 g			75								
Aretan	0.75 g				178*	238**						
Quintozene 20%	0.18 g	168*								132	100	
	0.35 g	161										
	0.70 g	139										
Tecnazene 40%	0.088 g	145										
	0.175 g	127										
	0.350 g	114										
TCTNB 5%	0.70 g	120										
	1.40 g	180*										
	2.80 g	182**										
Captan 80%	0.3 g		112									
	0.6 g											134*
Tuzet	0.5 g		137									
Urbacid 80%	0.1 g		100									
	0.2 g		108									
Rhizoctol 10%	0.25 g		129									
	0.50 g		113									
Benquinox/thiram 5/50%	0.38 g		137					138	173**			95
	0.75 g		163**									
Benquinox 10%	0.1 g		90									
	0.2 g		94									
Thiram 10%	1 g						150					
	2 g						238**					
	4 g						263**					
	6 g						259**					
	8 g						224**					
	10 g						264**					

to control phycomycetes in the soil effectively (allylidene diacetate: SCIARONI and MCCAIN, 1963; dexon: ZENTMEYER, 1967).

4.4.7. *Organic amendments*

Apart from some treatments already discussed (apple roots, section 3.3.5. and phloridzin, section 3.3.6.) a few other organic materials were applied in pot experiments.

Ground industrial residues of soybeans, coconuts, and oilpalm kernels were mixed into the soil. The major constituents of the products and the results on growth of apple seedlings planted 5 weeks after the treatments are shown in table 43.

TABLE 43. Effect of industrial oil press residues on growth of apple seedlings. (*cm* and %)

Treatment	Composition of amendments (%)			Dosage (per 1 of soil)	Growth
	fat	albumen	fiber		
O					*4.3* 100
DD				0.2 ml	95
CP				0.2 ml	251**
Pressed soybean residue	1.5	40	5	5.0 g 20.0 g	123 186**
Pressed coconut residue	6	20	14	5.0 g 20.0 g	118 221**
Pressed oilpalm kernels residue	6	16	14	5.0 g 20.0 g	137 202**

There is some effect of the treatments, but only in high dosages, and with little differences between the products. None of the three therefore is very effective in controlling SARD.

In another experiment, chitin was applied as milled shrimp shells, of which it is a major constituent (sample kindly provided by Dr. J. van der Spek). Data from the literature show that it is effective in controlling soil fungi, by the stimulation of organisms feeding on fungal cell wall material (BUXTON, et al., 1965; MAURER and BAKER, 1964; MITCHELL, 1963; VAN DER SPEK, 1968). In our experiment various dosages of this material had no effect on growth of apple seedlings.

4.4.8. *Heat*

In pot experiments, heating the soil for 1 hour to 120 °C and for two hours to 60 °C, were as effective as chloropicrin in controlling SARD (section 4.2.). In section 4.6. the effect of the latter heat treatment will again be shown for a range of cases. In the field, steaming the soil was applied once (section 4.3.3.) with very good results. Also in the literature (ANONYMOUS, 1962a; BOLLARD, 1956) there is general agreement on this point.

Figure 12 shows the results of two pot experiments with the 'M' soil from the river clay area. A range of temperatures was maintained for 30[3] and 120 minutes respectively. There is an indication of some effect of exposure time, but with 60 °C the control is very good in both cases. At 45 °C, and 30 minutes exposure time, the nematodes (the infestation level in the control soil was low) were all killed.

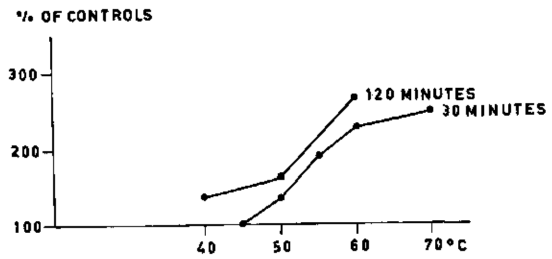


FIG. 12. Effect of heat treatment of apple ('M') soil on growth of apple seedlings in pot experiments.

The experiments confirm the beneficial effect of a typical broad spectrum treatment, and also that the elimination of nematodes does not lead to control of the disease.

4.5. Conclusions on sections 4.3 and 4.4

The data in sections 4.3. and 4.4. demonstrate that SARD can be effectively controlled by treatments which have a broad spectrum effect on soil microflora and fauna. High dosages of DD were as effective as heat and CP in controlling the disease. In pot experiments, propylene oxide, thiram and organic mercury compounds were also effective.

With various fungicides the results were poor or moderate. Formalin and CBP had moderate effects which is in accordance with data from the literature indicating their moderate action as broad spectrum soil fumigants. Methyl bromide, tested in field experiments, about equalled CP; it was applied at a high rate (100 g/m^2). In England poor to moderate results were reported with lower dosages (PITCHER et al., 1966).

Nematicidal treatments (DD at normal rate, ethylene dibromide, temik, and heat 45 °C 30 minutes) and the MIT group of compounds which have strong fungicidal activities, failed to control SARD.

As yet, apart from some effect of very high dosages, organic amendments have not proved to be effective, which may be considered another illustration of the persistence of the disease. Testing of other organic materials, however, should not be discouraged by these results, because it is possible that other materials, not tested so far, are more specifically active against the causal factor of SARD.

[3] I thank Drs. G. J. Bollen, of Lab. of Phytopathology, Landbouwhogeschool, for carrying out the 30 minutes heat treatments with air/steam mixtures.

4.6. Development of an advisory method

4.6.1. *Introduction*

An essential part of any control programme is to know when it is necessary to take measures. This is particularly evident in the case of controlling SARD, because the treatment with chloropicrin is costly and the disease does not occur on all apple soils (VAN MARLE, 1962).

The condition of the old plantation offers no insight into the occurrence of SARD, and thus cannot serve to predict occurrence of the disease in a following planting.

The results with preliminary experiments in pots of different sizes, using both seedlings and rootstocks, poor growth caused by SARD could be reproduced. This prompted research into the possibility of using a pot test for advisory work.

The use of apple seedlings for this purpose was first mentioned by SCHANDER (1958), who suggested to plant seedlings in the field to be replanted and to conclude from their condition about the presence of SARD. Interesting results were obtained by this author when seedlings were grown in an apple orchard, and on adjacent fresh land. The following year apple was again sown, also on new adjacent plots. In this experiment it was shown that SARD could be demonstrated by differences in growth between plants on fresh and apple soil, and also by different degrees of growth reduction in the second year, in accordance with the crop sequences in the preceding years. It could be shown that the symptoms were not related to nematodes present. In another of SCHANDER's experiments specificity was apparent; when growth on fresh soil was compared with growth on apple, pear and cherry soils, growth depressions were strong, moderate and negligible respectively.

The large-scale use of pot tests in advisory work in connection with a soil-borne disease is reported by SHERWOOD and HAGEDORN (1958). The cause of the disease is *Aphanomyces euteiches*, which is responsible for poor growth of pea in many cases of repeated cultivation of this crop. The direct evaluation of the pathogen in the soil is not possible. In a pot test, however, at a certain stage, the condition of the root system is used as a basis for advice on the possibility of growing pea on land previously cultivated with this crop.

Cherry seedlings were found not to be satisfactory planting material for use in a routine pot test for advice with regard to specific cherry replant disease (ANONYMOUS, 1968b).

Investigations into the applicability of a pot test as a basis for predicting occurrence of SARD were carried out in co-operation with the Bedrijfslaboratorium at Oosterbeek.

4.6.2. *Materials and methods*

Two series of pot experiments were carried out in 1962 and 1963. Pots of 750 ml were buried in the soil. In 1962, all were left in the open, in 1963 the tests were carried out in duplicate; one of each was left in the open, the other was grown under glass (Dutch lights). In 1962 the effect of nematicidal (DD) and broad spectrum (CP, heat) treatments was tested on both apple and fresh soils. In 1963 only DD and CP treatments were carried out. There were 6 replicates of all treatments. To cancel out the nitrogen effect of the chemical treatments and heat, and to prevent deficiency diseases, the usual complete fertilizer mixture, rich in nitrogen, was administered two weeks after planting. In both years, samples from field experiments were included for comparison of effects in the field and in the pot test. Also, in 1962, three different kinds of planting material were compared.

In 1962, there were 58 samples, all from the river clay area. There were 46 apple soils and 12 fresh soils. Ten samples from field experiments (1 fresh soil) were taken in triplicate to allow for the testing of the three types of planting material, Bittenfelder seedlings, and M.IV rootstocks and root cuttings, on all these ten soils. Of the other 48 samples, 40 (9 fresh soils) were grown with seedlings, and 8 (2 fresh soils) with rootstocks.

In 1963, there were 32 samples. There were 18 samples from the river clay area (2 fresh soils) also tested in 1962. Of these 9 were from field experiments, which were not the same as in 1962. There were also 6 (1 fresh soil) from the Bangert area, and 8 (1 fresh soil) from the South Western part of the country.

In the laboratory the soil was thoroughly mixed and 4 liter portions were made for the treatments and the controls. The remaining soil was used for chemical analysis and estimation of the nematode populations. The fumigation treatments (DD: 0.2 ml, CP: 0.2 ml/l of soil) were carried out in plastic bags (polyvenylchloride of 0.2 mm thickness). The bags were kept closed for two weeks.

For the heat treatments the soil was kept at 60°C for two hours in flat metal containers in a water bath[4].

The seedlings were, as usual, of the selection 'Bittenfelder'. The M. IV rootstocks were obtained from a commercial nursery, and consisted of selected small sized layers from stoolbeds. The stem diameter was 4–5 mm. The root cuttings about 3 cm long, also M. IV, were prepared in January 1962. They rooted well and formed some leaves before planting.

In 1962 measurements were taken 52, 67, 81 and 102 days after planting; in 1963 at 42, 52, 63, 77 and 141 days. With seedlings total length was recorded and with rootstocks and root cuttings the length of the new shoots.

4.6.3. *Growth on fresh and apple soils*

The reproduction of SARD under the conditions of the pot tests is the basis for further discussions of the results. Therefore reproduction of disease is discussed briefly in this section. The same criterium will be applied as in section 3.2., viz. the comparison of growth on treated and untreated fresh and apple soils.

Data will be presented which apply to the 1962 series of tests, with apple seedlings. There are 40 apple soils and 7 fresh soils which can be used for a comparison.

[4] Thanks are due to Mr. S. Stemerding, of P.D., Wageningen, for carrying out these treatments.

TABLE 44. Average growth of apple seedlings (cm/plant) and average of relative growth (% of controls) on 7 fresh soils and 40 apple soils. Growth recorded on 67th day after planting.

Treatment	Dosage	Fresh soils		Apple soils	
		cm	%	cm	%
Control		10.5	100	7.2	100
DD	0.2 ml/l of soil	11.2	105	9.3	130
CP	0.2 ml/l of soil	13.2	131	17.3	261
Heat	60 °C, two hours	13.7	134	16.6	245

The data are summarized in table 44.

The table shows better growth of seedlings on untreated fresh soils than on untreated apple soils, and a much stronger effect of radical treatments on apple soils than on fresh soils. This indicates that SARD is reproduced under the conditions of the experiment, which is further supported by the moderate effect of DD on apple soils. The differences between fresh and apple soil are the more striking when it is realized that the figures given for apple soils apply to a range of cases including soils not or only slightly infested.

In 1963, there were only 4 samples of fresh soils. With regard to the comparison of growth on fresh and apple soils, and the effects of the treatments, the results were comparable with those in 1962.

4.6.4. *Evaluation of techniques*

4.6.4.1. Time of taking measurements

It had already been noticed in earlier experiments in pots of the same size that growth differences (effects in %) between controls and successfully treated pots passed a maximum. At planting there is no difference and after three to four months differences on an average decrease probably as a consequence of the fact that the size of the pots becomes a limiting factor first for the best growing plants.

Evidently the best time of evaluating the experiments is the date of recording the maximum relative growth difference between radical treatments and controls. Table 45 gives information about dates on which maximal differences were

TABLE 45. Apple soils of 1962 pot trials. Number of cases with maximal effects at the 4 ages (days) of recording growth. Root cuttings were not measured when 81 days old.

Planting material	DD				CP				Heat			
	52	67	81	102	52	67	81	102	52	67	81	102
Seedlings (Bittenfelder)	19	14	5	1	10	24	4	1	13	21	4	1
Rootstocks (M. IV)	5	2	3	7	2	3	6	7	2	3	3	10
Root cuttings (M. IV)	3	2		3	0	1		7	0	0		8

observed for the 1962 series of pot tests. If the differences were equal on more than one date, these were recorded as maximal for all the dates concerned.

The table shows that in most cases growth differences of seedlings were maximal at the second, and of rootstocks and root cuttings at the 3rd–4th measurements. There are, however, quite a few exceptions to these average dates. It is concluded that for the evaluation of a pot test several measurements have to be taken in order to establish the approximate date of maximum growth difference to be used as a basis for advice.

In the 1962 series maximal effects with fresh soils in general, and with DD treatments of apple soils, were more often observed at the first measurement. This suggests that side-effects not related to the control of SARD are more strongly expressed in early measurements. Probably this can be partly explained by the nitrogen effect, which will be strongest shortly after the treatment. Therefore too early an assessment of the results of a pot test is not recommended. The observations also indicate that the treatments were not phytotoxic under the conditions of the experiment.

In 1963 differences between dates of measurements were less pronounced than in 1962. There was a slight tendency for the effects to increase with later measurements. It is clear that seasonal variations may influence the effect and also the date at which maximal growth differences occur.

4.6.4.2. Types of test plants

In the foregoing section it was shown that seedlings reach maximum growth differences at an earlier date than rootstocks and root cuttings. This may be an advantage, but in comparing types of test plants, other criteria are still more important, particularly the actual growth and growth differences observed with the various types of planting material.

In 1962, 9 samples of apple soil were grown with all three kinds of planting material (table 46). It appears that seedlings show the greatest differences. With rootstocks the effects were much smaller; this seems to be due mainly to a

TABLE 46. Average growth of seedlings (Bittenfelder), rootstocks (M. IV) and root cuttings (M. IV) in pot tests with 9 apple soils, at 2nd and 4th measurement (age: 67 and 102 days respectively).

Planting material	Growth in % of controls			Growth in cm per plant			
	DD	CP	Heat	O	DD	CP	Heat
Seedlings							
2nd measurement	139	312	304	5.9	8.4	17.3	17.3
4th measurement	118	234	226	10.4	12.3	22.9	23.3
Rootstocks							
2nd measurement	115	142	131	14.5	16.7	20.5	19.0
4th measurement	117	148	140	15.8	18.4	23.3	22.0
Root cuttings							
2nd measurement	99	155	131	4.3	4.2	6.6	5.6
4th measurement	103	185	157	5.1	5.2	9.4	8.0

fairly good growth of the controls. It may be that food reserves present in the stem and roots are responsible for this. In addition it is possible that the pots are somewhat too small for this type of plant material, and growth of plants in treated pots would be affected most. In section 4.2. it was shown that very good results were obtained with M. IV rootstocks in 10 l pots. With root cuttings, the rather small effects are due to poor growth of many plants in the treated series, though in some cases the effects approach those observed with seedlings. Observations suggest that the rooted cuttings did not support the process of transplanting very well and many plants did not recover from the shock. The difference in behaviour of rootstocks and cuttings is not due to genetic differences, because both were Malling type IV. Anyhow, it is important to note that both show the same tendency as seedlings with regard to the effect of the treatments: there is markedly greater effect of the broad spectrum treatments heat and CP than of DD.

Since Bittenfelder seedlings, although known as rather homogeneous, still show a certain degree of variability, another apple selection, Graham's Jubileum, was compared with Bittenfelder seedlings. Both were grown on fresh soil, apple soil, and steam-sterilized apple soil. Table 47 shows that the results with both kinds of seedlings are about identical.

TABLE 47. Growth of Bittenfelder and Graham's Jubileum selections of apple seedlings on untreated and steam-sterilized apple soil and on fresh soil.

	Bittenfelder		Graham's Jubileum	
	cm	%	cm	%
Apple soil ('M')				
control	9.3	100	7.6	100
steam	22.0	237**	19.1	251**
Fresh soil	24.3	261**	22.4	295**
F-value	29.33		18.16	

Generally the impression was gained that factors other than genetic variability are largely responsible for the variations observed within groups of plants of one treatment, especially since variability among plants of type M. IV was also appreciable. Slight differences in planting material with regard to rooting, etc., and in the conditions early after planting are probably important causes of the later variations in older plants. The number of six replicates as applied in these series can therefore be considered a minimum.

4.6.5. *Comparison of results of pot tests and field experiments*

A most important aspect in relation to the suitability of the pot test for advisory work is the correlation of the results in the field with those from pot experiments. In section 4.6.2. it was already indicated that 19 different cases were tested simultaneously with trees in the field and seedlings in pot tests. In

1962 there were 9 cases of apple soils. In all corresponding pot tests, seedlings, rootstocks, and root cuttings were grown. In 1963 nine samples from other field experiments were grown with seedlings in pots placed in the open and under glass. These 9 cases had also been included in 1962 pot tests. In addition to these two groups, data on other comparisons of field and pot results were collected and a number of these will be discussed in this section.

Table 48 gives the data of the first group of 9 apple soils in 1962. In most cases of apple soils strong effects of CP treatments were observed.

TABLE 48. Comparison of results of field experiments (nos. 3–11, section 4.3.3.) and pot tests. Figures based on measurements of total length of seedlings and of shoot length of trees, rootstocks and root cuttings. Cases arranged according to effects with seedlings in pots (2nd measurement). Rootstocks and root cuttings: 4th measurement.

Field no.	CP: growth in % of controls				Growth in cm per plant			
	Field	Pots			Field		Seedlings in pots	
		seed-lings	root-stocks	root cuttings	control	CP	control	CP
3	605**	427**	174**	106	20	121	3.3	13.9
4	496*	420**	168**	181**	57	283	3.7	15.6
5	293**	411**	176**	232**	68	199	4.8	20.3
6	1053**	351**	153**	237**	36	379	6.5	22.8
7	214*	312**	150**	202**	139	298	7.2	22.3
8	336**	303**	135**	206**	100	336	5.6	17.1
9	198**	247**	125*	196	48	95	7.4	18.2
10	265**	182**	131*	212**	26	69	9.5	17.3
11	138	161**	121	92	60	83	4.9	7.9
Average	400	321	148	185	62	207	5.9	17.3

The figures demonstrate that with the exception of no. 11, the soils are moderately and mostly heavily infested. There is good correlation between results obtained with seedlings and with rootstocks, although the differences between the cases are much smaller with the latter, suggesting a less distinctive capacity of the test with the use of this planting material. The irregularity of the results with root cuttings has already been discussed above. The correlation with field results are not very clear, suggesting that degrees of disease severity within the group of strongly infested cases are not clearly indicated by the test. This is not surprising in view of the widely different conditions in the field, as is illustrated by the figures showing actual growth. These show a considerable variation in each column, except in case of the CP treated series in the pot experiments. Excepting no. 11, length of seedlings does not vary as much as the figures in the other columns. This suggests that CP treatment has resulted in rather uniform conditions in most of the different soils; this may be explained by the combined effect of two factors: 1. the soils are all of approximately the same type and 2. CP treatment has effectively eliminated SARD. The fact that growth on CP treated plots in the field varies more strongly illustrates that other

TABLE 49. Effect of chloropicrin treatment (growth in % of controls) on growth of apple seedlings with 17 soils from the river clay area in the 1962 and 1963 series of pot experiments, and on growth of trees in field experiments on 9 of these soils in 1963. Based on length of seedlings (maximal effects) and increment of stem circumference of trees. The figures for the apple soils (nos. 1–15) are arranged according to CP effects in the 1963 pot experiments in the open. Nos 16 and 17 are fresh soils.

Sample number	Pots 1962	Pots 1963		Field 1963
		in the open	under glass	
1	294**	387**	297**	
2	326**	311**	321**	
3	544**	305**	240**	152
4	302**	273**	159**	
5	464**	253**	315**	358**
6		216**	181**	410**
7	274**	203**	171**	391**
8	198*	184*	261**	
9	186**	168*	161**	177**
10	345**	168*	158**	209**
11	321**	160**	161*	198*
12	165	157	155	
13	176**	153	163*	
14	225**	149	183**	168*
15	157**	142	133	174**
Average of apple soils (not including no. 6)	284**	215**	204**	
16	123	195**	121*	
17	114	134	109	

factors conditioning growth show greater variation in the field than in pots.

Table 49 gives information on the second series of comparisons made in 1963. The same table gives the figures for the other cases which were tested both in the 1962 and 1963 pot experiments (soils from the river clay area).

The two fresh soils (16 and 17) show the least stimulation by the chloropicrin treatment. With soil 16 in pots in the open growth of plants in CP treated pots was 195%. This observation was made at the first measurement; later measurements gave much smaller effects and on the 77th day, at which the effect was maximal for most other soils, growth was only 140% of the control.

In 1962 the effects were mostly stronger than in 1963. The tendency of the effects to correlate with those of 1963 is rather clear, except for sample numbers 10 and 11. Correlation between trials in the open and under glass in 1963 is also rather clear, again with two exceptions, nos. 4 and 8.

With regard to the correlation with field results, no. 3 which shows much less effect in the field than in the pot experiments, seems to fit in least of all cases. The reason for the poor field results was a very low standard of cultivation causing very poor growth of the trees on treated and untreated plots. In the second year the orchard was tended much better, and growth of the trees on the chloropicrin treated plots was 350% of the controls. This indicates that the strong infestation as indicated in the pots, was indeed present also in the field.

In later years pot experiments were taken with soils from various field experiments. The figures which may serve to compare results of soil disinfection in the field and in pot tests are shown in table 50.

TABLE 50. Effects of CP treatments in pot tests and in field experiments (% of controls). Based on measurements of total length of seedlings and of length of new shoots of trees.

No.	CP effects		
	Pots	Field	
		1st year	2nd year
1	291**	131	280**
2	255**	273**	299**
3	249**	252**	197**
4	247**	241**	166
5	231**	226**	
6	210**	219**	
7	200**	188**	
8	156	173*	184**
9	141	133	125
10	127	138	137

From this table it can be seen that, generally, there is good agreement between results of pot and field experiments. Numbers 8, 9 and 10 are typical cases of moderate and low infestation, as is shown in both the pot tests and the field experiments.

4.6.6. *Discussion*

The two main points which make it interesting for the grower to send in samples for a pot test are:

1. Treating the orchard for specific apple replant disease is a costly affair.
2. Replant disease is not always present, especially not in certain areas.

If replant disease was uniformely distributed over the whole fruit growing area, or if the treatment was cheap, there would be no need for an advisory method.

It should be realized that in applying the pot test two kinds of financial risks are involved: 1) the chance that an advice is given not to treat while in reality treatment would have been necessary, and 2) the reverse situation, that is, to advise treatment while it is not really necessary. The relative importance of these two considerations depends on economic factors – especially the cost of the treatment and the expected return. This latter factor can only be safely judged if experience on the effect of the treatment is gained over a period of years in the productive phase of orchards. This information is lacking, because orchards included in the experiments and commercially treated orchards are too young. Therefore, at this stage of our knowledge, it was decided to avoid at any rate the second risk and, not to advice growers to treat unless it is very clear that treatment is indeed justified.

The first obvious possibility of the pot test is the distinction between fresh soils and apple soils. Fresh soils also react favourably to the treatment with chloropicrin, due to the 'nitrogen' effect and to the elimination of organisms which are mildly harmful to apple root systems (cf. DOMSCH, 1963a). The data presented in table 51 indicate that a growth on CP treated soil up to 150% of controls may occur on fresh soils ('unspecific response', PITCHER et al., 1966). This is therefore adopted as a safe margin of classifying a soil as infested.

TABLE 51. Growth of apple seedlings on DD and CP treated fresh soils in % of controls.

Treatments	Soils no.								
	1	2	3	4	5	6	7	8	Average
DD	98	102	124	113	111	107	139	101	112
CP	114	161	107	114	110	154	165	115	130

The data in tables 48, 49 and 50 indicate that the effects in pot experiments mostly do not 'exaggerate' the improvement of growth of the trees in their first year of growth. The figures also indicate that different degrees of infestation are shown in the pot experiments, but that no very strong correlation exists between results in pot trials and in the field. This is not surprising as many factors interfere with growth and growth differences in the field: soil conditions, standard of cultivation, varieties, rootstocks. All these factors influence the results of field experiments. Also climate interferes with pot tests less than in field experiments.

It was decided when giving advice not to suggest too close a correlation between results in the pot tests and improvement of growth after CP treatment in the field to the growers. The figures in tables 48–51 suggest that three categories should be distinguished: no appreciable SARD present if growth is below 150%; treatment with chloropicrin highly recommended if growth is 200% or more, and the intermediate category of growth between 150 and 200% of controls. In the latter category the condition of the untreated plants is taken into account. If growth of untreated plants is good, and the general condition of the plants does not suggest the presence of SARD, the grower is informed that replanting without treating the soil will not present great difficulties, although treating the soil with chloropicrin would probably be profitable. In the other cases the condition of the untreated plants strongly suggests that SARD is the cause of the growth differences observed. Hence the grower is advised to apply soil fumigation with chloropicrin since the field is moderately infested with SARD and soil fumigation would therefore be economic.

To give the grower a better idea of the results of the pot test, the 'Bedrijfslabora-

BEDRIJFSLABORATORIUM VOOR GROND- EN GEWASONDERZOEK

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CONS.	ORG.

Monster van: De Heer H.H. Appel, Fruitmalsen

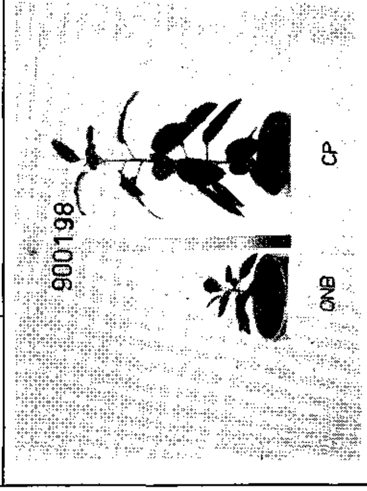
Voor rekening van:
De Heer H.H. Appel
Schotweg
Fruitmalsen

Het totaal van de onderzoekingskosten bedraagt: f

STORTEN OP POSTREKENING NR [illegible] VAN BEDRIJFSLABORATORIUM VOOR GROND- EN GEWASONDERZOEK TE OOSTERBEEK, ONDER VERMELDING VAN →

BETALINGSNUMMER	AANTAL MONSTERS
W 900198	1

Onderzoek nummer	Perceelsaanduiding	Gemiddelde lengte van de zaailingen in cm				Groeiverbetering in % na behandeling met		
		Onb	na behandeling met					
			DD	CBP	CP	DD	CBP	CP
w900198	Blok 1	5,3			15,5			192



Gemiddelde groei in Uw monster

Toelichting:

Op dit perceel is sprake van zeer ernstige bodemmoeheid.
Bij herinplanting kan hier zeer sterke groeiremming voorkomen, zodat het noodzakelijk is een grondontsmetting uit te voeren.
Zie voor verdere toelichting de bijlage.

VF 31-1500-27-7-66-M

Aanvragen om adviezen worden alleen behandeld op voorwaarde, dat de aanvrager afstand doet van ieder recht op aansprakelijkheid van het te geven of gegeven advies.

FIG. 13. Form as used to inform growers of the result of the pot test carried out by the 'Bedrijfslaboratorium'. The photograph sticked on the form shows growth of average seedling of each treatment.

torium' provides a photograph of the average plant of each treatment on the form which also gives the results in cm and growth improvement in % over the controls, and the written advice (figure 13).

The results shown in table 49 suggest that the effects observed may differ from year to year, and consequently that the interpretation of the figures for advisory work also has to be adapted each year. This should indeed be done, in principle, but four years of advisory work (KLEIJBURG, personal communication) have shown that so far the above mentioned criteria can generally be maintained. Uniform conditions – also of the soil surrounding the buried pots – are required. The plants are grown under glass. Table 49 does not show that this was an advantage in the year the influence of the glass cover was tested, but it was found that the risk of unfavourable weather conditions could not be taken.

In a few cases it proved to be impossible to give an advice, namely when plants in both control series and in the chloropicrin treated pots grow poorly. The cause is poor soil structure.

Structure of the soil is sometimes deteriorated as a result of transportation, mixing, and potting. Soils from certain areas, for instance the North East Polder are particularly unstable in this respect. Careful treatment and avoiding excessive watering have proved to be rather effective in preventing these difficulties.

At the Bedrijfslaboratorium, where yearly about 80 samples are sent in, a new system of watering has been adopted in 1968. Water is given by means of a permanent dripping system, which is placed between the pots. (KLEIJBURG, personal communication) Thus the surrounding soil – a mixture of sand and peat – is kept moist. There is sufficient drainage of excess water. With this system moisture conditions are the same for the soil in all pots, also when plants in CP treated soil will need more water than the controls. The pot test is now also being used in England (SAVORY, 1967), Belgium (GILLES, 1968) and South Africa (GILIOMEE, private communication). Good correlations have been reported from Belgium and England, but it was also stressed (SAVORY, 1967) that especially in the intermediate category, results should be interpreted with caution.

4.7. NOTES ON THE APPLICATION OF CHLOROPICRIN IN PRACTICE

The first commercial application of CP, on a field of about 1 ha, took place in November, 1962. From the autumn of 1963 onwards the application of CP was officially recommended. In most cases the product is applied in late summer and autumn. In 1963 7 ha were treated, in 1964 20 ha and in 1965 40 ha. In the following years the figures were also about 40 ha.

Strips of 150 cm wide are treated at the site of the row which has to be planted later. From the foregoing chapters it will have become clear, that the trees should be given a good start in the first year, and it was therefore not found necessary to treat the whole field, or strips wider than 150 cm. Strips somewhat less wide would perhaps also be sufficient, but no experiments were done to test this, mainly for practical reasons. With tractor drawn machinery small deviations from the planned site of the treatment are difficult to avoid. With narrow strips, the chance that trees will later be planted off the treated land will be much increased.

There is always a plastic cover used, which is now obligatory. In comparisons of a plastic cover and a water seal made in field experiments, both were about equally effective. This illustrates that there is no risk of recontamination. This is also substantiated by the immobility of the disease (THOMPSON, 1959) and the soil mixing experiments, which illustrated that even if some inoculum is present, no strong re-establishment of disease may be expected.

The application of a water seal will normally be difficult to achieve technically, because of the large quantities of water needed in a short time.

With regard to the plastic sheet used, polyethylene of 0.02 mm proved to be effective. In experiments on light soil a complete nematode kill till 30 cm depth

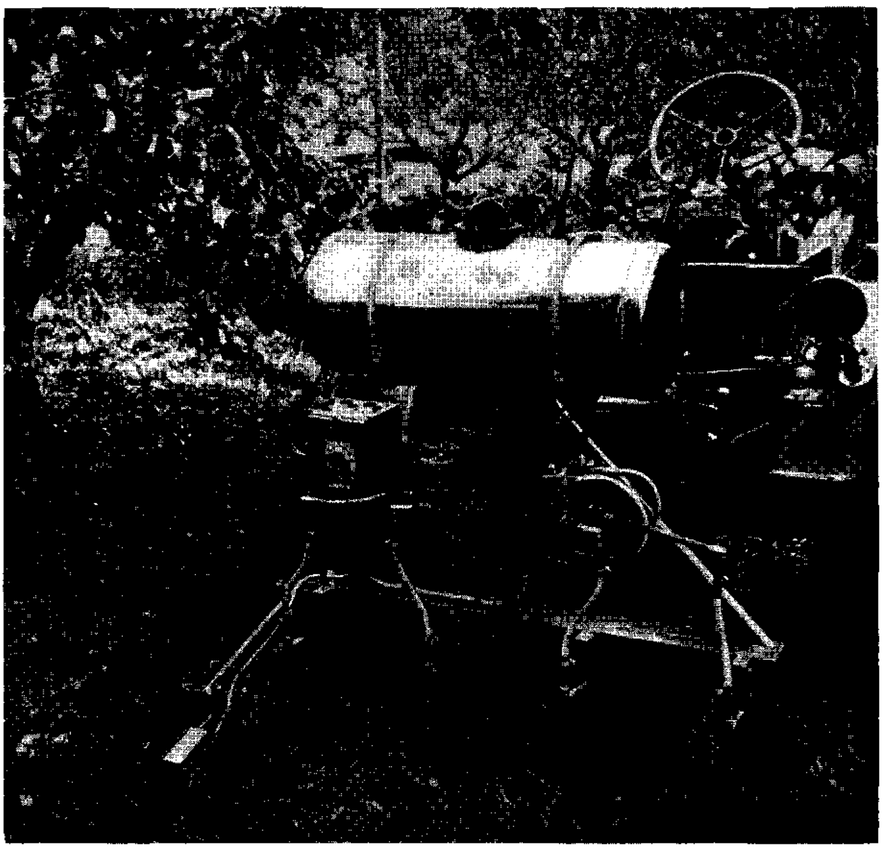


FIG. 14. Fumigating machine used to treat orchard soils. The picture shows wheel which ensures dosage independent of speed, four magnetic valves, and four chisels by which fumigant is injected.

in the soil was achieved when CP was placed in small dishes under a cover of 0.02 mm polyethylene. This demonstrates the efficiency of this kind of plastic in sealing CP. The importance of an effective seal is, moreover, generally recognized (GODFREY, 1934; GODFREY et al., 1934; OVERMAN et al., 1965; YOUNGSON et al., 1962; WILHELM, 1961). The application of a plastic cover is obligatory for safety reasons: if CP could escape from the treated land rather quickly, it could do much harm to crops on adjacent fields, to animals, and to man. A few cases in which damage to crops was reported (apples and vegetables) illustrated that quick laying of the plastic is important to avoid these risks. Apple trees react already to very low concentrations of CP by dropping their leaves.

With machinery used by private contractors, CP is introduced in the soil by chisels 25 cm apart, to a depth of 20–25 cm. Two types of machines have been used so far. One is based on the gravity flow principle, with magnetic valves which are regulated automatically and depend on a little wheel which follows the machine. Thus dosage is independent of speed (fig. 14). With the other machine, the fumigant is kept under constant pressure (fig. 15). Dosage is regulated by taps and the speed of the machine. Plastic is laid also by machinery (fig. 16). In the first case a separate machine is used, and in the second, the plastic laying apparatus is built into one unit together with the fumigator. All the mentioned machines are of a rigid construction for use on heavy soils.

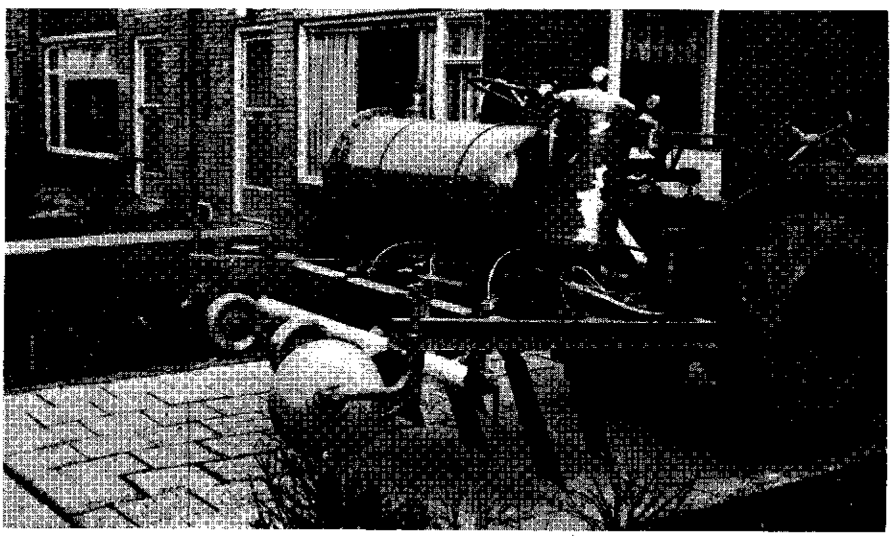


FIG. 15. Fumigating and plastic applying machine for treatment of orchard soils. The machine is based on a constant pressure system.

Prior to fumigation, the soil should be brought into seedbed condition. The impression was gained, by successful treatments even in mid winter, that low soil temperatures are not a serious drawback in CP application. A pot experiment was carried out in which the soil, at a rate of 0.2 ml/l, was treated at three different temperatures. The soil was brought at the desired temperatures prior to fumigation and kept at constant temperatures during the 7 days exposure period and during escape of CP. At 3, 12 and 18 °C growth of CP was 213, 249 and 251 % of controls respectively, which confirmed that good effects are possible at low temperatures.

Studies of nematode populations after treatment showed that generally good penetration of CP in the soil was achieved to a depth of about 50 cm.

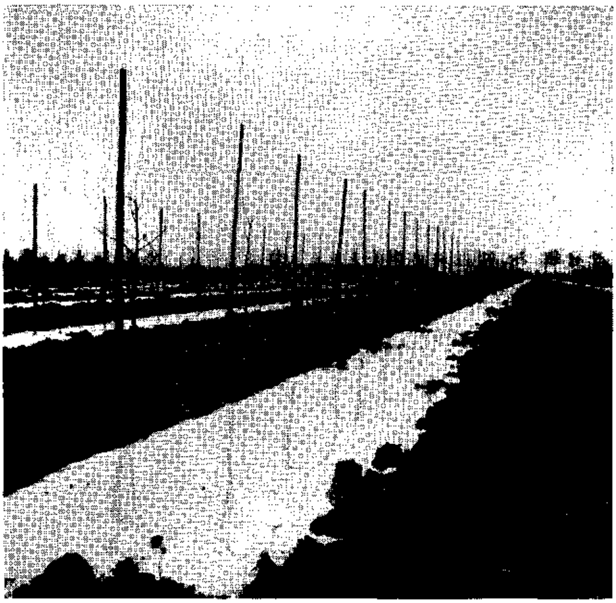


FIG. 16. Strip of land treated with CP, and covered with a polyethylene sheet by machinery.

It is advised not to remove the plastic sheet when wheather conditions may be expected to allow CP vapour to drift in an undesirable direction.

To avoid phytotoxicity to the new apple plantation, spring application is dissuaded, though there have been successful treatments in Febrary and March. In cases of doubt, the growers are recommended to apply the 'Lepidium' test. When seeds of *Lepidium sativum* are brought together with a soil sample in a closed container, seeds will not germinate if traces of CP are present.

5. GENERAL DISCUSSION

5.1. Introduction

In this discussion, data from all the foregoing chapters, as well as data from the literature will be used. Literature references will be mostly confined to articles either applying to SARD, or of special interest in making comparisons with related problems, the more so because a full and recent review of literature on specific replant diseases of perennial crops is available (Savory, 1966).

Some aspects have already been discussed in some detail, such as the role of nematodes (chapter 2) and the advisory method (section 4.6.) so that these will only be referred to rather briefly.

The most important section of this chapter is the discussion of possible causes of SARD, since this forms the central and most fascinating problem. An attempt will be made to consider the available data as much as possible from the viewpoint of their value for giving indications of the possible cause. We shall discuss the possible categories of causes, i.e. soil structure, deficiencies, toxins and organisms, as has been done by other authors (Klaus, 1940; Fastabend, 1955; Savory, 1966).

5.2. Possible causes of specific apple replant disease

5.2.1. *Requirements*

It is convenient first to list the symptoms, characteristics, and other observations, most of the latter being based on experimental results, in order to indicate the requirements to be met by any causal mechanism. The term 'causal mechanism' is used because it comprises all possibilities: parasitic and non-parasitic factors, single and multiple causes, complex diseases.

The causal mechanism should fit in with, or at any rate not be contradicted by the following characteristics:

a. symptoms above ground: poor growth with few or no specific characters,
b. symptoms of the root system: epidermis and primary cortex of young roots affected, few root hairs formed, premature decay of these tissues, stele not necessarily attacked. Bacteria and actinomycetes present but mostly confined to outer tissue layers. Older roots not visibly affected,
c. non-lethality; in the field early poor growth followed by recovery starting in 2nd or 3rd year,
d. persistence, irrespective of intervening crops,
e. specificity,
f. recovery after transplanting to fresh soil,
g. maximum disease intensity found rather superficially in the soil,
h. failure of transmission by grafting,
i. failure of transmission by adding roots to soil,

j. moderately strong growth reduction (disease) when 10% of diseased soil is mixed with fresh soil,
k. failure to induce growth reduction by mixing phloridzin into the soil,
l. addition of leachates from apple soil to fresh soil not leading to disease,
m. strong positive effect on growth of plants in apple soil by high soil moisture conditions,
n. strong favourable effect of low soil pH, and acidification of near neutral soil; colour of root system affected: in acid soil pale yellow, in neutral soils reddish brown,
o. no effect of soil temperature (only one experiment),
p. disease not controlled by addition of nutrients, and minor elements,
q. good control by broad spectrum treatments (by chemicals and heat),
r. limited effect of nematicides, having little influence on soil flora,
s. limited effect of methyl isothiocyanate and related products.

Two observations will not be discussed further in the following sections, and will therefore be commented upon here. There was (section 3.3.4.) no effect of nutrient solutions from mist cultures of apple; from this observation little can be concluded, because either the disease is not transmitted by these solutions, or it does not occur under the conditions of a mist culture.

Secondly, although the effect of gibberellic acid as shown in section 3.3.11. is very interesting, because the experiment confirms that SARD primarily affects the root system, the experiment does not point to any specific category of possible causes.

In order not to make the list too complicated, some minor observations, to which reference will be made in the following sections, have not been included.

5.2.2. *Poor soil structure*

The apple is very susceptible to poor soil structure, and the resulting growth reduction resembles the symptoms of SARD. Non-lethality, recovery after transplanting, and failure of transmission by grafting, also do not contradict the possibility of poor soil structure being a causal factor, but other requirements very clearly show that poor soil structure can be excluded.

Specificity, the effect of mixing soils, and the effect of broad spectrum soil disinfectants cannot be explained if poor structure is the cause of SARD. Furthermore, there is no correlation between structure and disease as observed in the field; SARD may often be particularly severe in many soils with a good structure. The correlation of results in field and pot experiments also leads to the same conclusion: the original structure of the soil in the field will not generally be conserved in the pots after the process of taking samples, sieving, mixing and putting soil in the pots. There is also general agreement among authors who considered the different possible causes of specific replant diseases of perennial crops, that poor soil structure (physical deterioration) is not the cause of any of these diseases (SAVORY, 1966; MARTIN and TSAO, 1968).

5.2.3. *Nutrient deficiencies*

Although poor growth may suggest that nutrient deficiency plays a part, the symptoms are not typical of any of the described deficiency diseases of apple (MULDER et al., 1963). The rosette formation as observed especially with seedlings, somewhat resembles symptoms of zinc deficiency, but the characteristic chlorosis of zinc deficient plants has not been observed. Further, adding a complete fertilizer mixture to apple soil has no effect, and no relationships have been found in the chemical composition of plant or soil, and disease incidence. In field experiments, Mg-deficiency has been observed occasionally on control trees and not on healthy trees. This is no doubt a secondary phenomenon. It is, indeed, surprising, that even in cases of strong growth reduction very few deficiency symptoms have been found, since, in other plant diseases, deficiency symptoms are rather frequent as secondary phenomena.

Although some requirements would fit the deficiency hypothesis, such as recovery after transplanting, non transmissability by grafting, and effect of low soil pH (which favours availability of many elements to the plants) there is much evidence that a direct deficiency is not the cause. Specificity is a character which is very difficult to associate with a deficiency. Beets for example grow very well on apple soils and are known to be very susceptible to boron deficiency, yet SARD has once been attributed to boron deficiency (KOBERNUSZ, 1951; HOCHAPFEL, 1952).

Soil fumigation and heat treatments are known to raise the availability of certain elements to plants (ALDRICH and MARTIN, 1952; DAVIDSON and THIEGS, 1966). But broad spectrum soil sterilants controlled the disease under a variety of soil conditions, which suggests that the correction of a deficiency was not the common factor in all these cases. VON BRONSART (1949) attributed the disease to a deficiency of minor elements, especially manganese, and the beneficial effect of soil fumigation to the liberation of Mn and other elements for the plants. In our experiments propylene oxide was an effective treatment, and the product is known to have little effect on Mn in the soil (ALDRICH and MARTIN, 1952). Nitrogen is the only element subject to side-effects from practically all the quoted treatments under all conditions, but it was shown that adding nitrogen to the soil alone does not control the disease. Also there was no relation between nitrogen side-effects and disease control, as was shown by products which did not control the disease, but are known to have nitrogen side-effects (DD, MIT).

The addition of 10% apple soil to healthy soil led to a marked growth reduction. This observation cannot possibly be explained on the basis of the deficiency hypothesis.

As in the case of poor soil structure, there is general agreement in recent publications that nutrient deficiencies are not the cause of SARD, or of other specific replant diseases.

Of course, in certain cases, both deficiencies and poor structure may contribute to damage by acting as stress factors.

5.2.4. *Toxins*

Toxic compounds have often been supposed to cause replant diseases. From their work, KLAUS (1940), FASTABEND (1955) and SCHANDER (1956), concluded that SARD is most probably caused by toxins, though no definite proof was given by these authors. The character of the disease more or less suggests a kind of 'intoxication' of the soil, and some of the experimental results by FASTABEND (1955) indeed pointed this way as the disease seemed to be transmitted by leachate from apple soil. He used only one apple soil, however, and nematodes as well as other organisms and other factors not related to SARD may have played a role. This author was not aware of the possible importance of nematodes.

The experiment described in section 3.3.3. strongly suggests that SARD is not readily transmitted by soil leachates. Moreover, transmission by soil leachates is most unlikely in view of the persistence of the disease. If the causal toxin is so easily washed out of the soil, persistence would not occur. A growth-depressing effect of apple roots mixed into fresh soil was also occasionally observed, but not generally and our own results also showed that adding apple roots cannot be considered a means of introducing SARD into fresh soil. Apple roots further had a tendency to improve growth on apple soils (section 3.3.5.). It was also shown that felling trees does not lead to such strong growth depressions as grubbing (ANONYMOUS, 1966b, 1967b).

Living apple roots are known to produce exudates rather abundantly, (ROGERS and HEAD, 1966) but in the soil these exudates will be mixed with other compounds, especially products from decomposing cortical cells and whole rootlets, and therefore, at present, there is little sense in discussing these factors separately. There is, however, reason to stress the effect of living apple roots, which produce exudates, since their presence is apparently connected with the induction of SARD into the soil.

Although the requirements listed in section 5.2.1. make it difficult to conclude with certainty on the toxin hypothesis, it seems most unlikely that a toxin is the direct cause of SARD. This would imply the existence of a compound which is persistent, specific, affected by soil pH, not associated with cut apple roots, and not being phloridzin or one of its breakdown products.

The beneficial effect of a range of broad-spectrum soil disinfectants and of heat also contradicts the toxin theory, but this leaves the possibility of a complex relationship between an organism and a toxin. It again seems improbable, however, that a toxin would persist in quantity in the soil, whether or not involved in a complex relationship. Further, if a persistent toxin was produced by the apple root system, then an accumulation would be expected, which is not supported by the recovery of trees after the first or second year.

Though the quoted observations suggest that a toxin is not the cause, it is also clear that nevertheless certain compounds of apple roots can be expected to play a role in the disease etiology, in connection with organisms. Since phloridzin is an important constituent of the apple root cortex (BÖRNER, 1961) and has been studied by several authors, it is interesting to discuss its role in some detail. The compound, a glucoside, is known to be strongly active in wheat coleoptile tests (HANCOCK et al., 1961; SARAPUU, 1964, 1965) and to play a role in the relation of apple leaves and their pathogen, *Venturia inaequalis* (NOVEROSKE et al., 1964; RAA and OVEREEM, 1968; RAA, 1968). BÖRNER (1959) showed that phloridzin inhibited growth of apple seedlings in water cultures, but no attempt was made to demonstrate the specificity of the effect on apple. His experiments were repeated (ANONYMOUS, 1962b) with the same result, but it was suggested that the effects were due to competition from the many bacteria which developed in the phloridzin-treated series. BÖRNER (1960), showed that under natural conditions a toxic concentration is not reached, and, moreover, that phloridzin is rapidly broken down in the soil.

The addition of phloridzin to soil did not reduce growth of apple seedlings (COLBRAN, 1953; ANONYMOUS, 1962b), and in our own experiments there was even some stimulation when added to apple soil. This is interesting also in view of the stimulating effect of apple roots when added to apple soil, which may be based on the presence of phloridzin in these roots.

Some of the breakdown products of phloridzin are rather strongly bactericidal and fungicidal (RAA, 1968, and personal communication), and an effect of these breakdown products may be expected to occur when phloridzin comes into the soil from decaying root tissues. The stimulation of growth on apple soils by cut roots and phloridzin may both be related to this biocidal effect. The phloridzin content of the root is known to be higher when little nitrogen is available (VIRTANEN and OLAND, 1954; HUTCHINSON et al., 1959; JÜRGENS, 1967). Ample nitrogen does not in the least control SARD, and this also could be interpreted as an indication that phloridzin or its breakdown products suppress the disease to some extent.

Effects of phloridzin breakdown products may also be related to soil pH. Roots grown in acid soil are of a pale yellow colour and roots in near neutral soils are reddish brown. Results of RAA and OVEREEM (1968) strongly suggest that differences in the pathways of breakdown of phloridzin are the cause of the observed colour differences. Breakdown may start by two different reactions. Hydrolysis to phloretin is maximal at pH 6.5. and oxidation via 3-hydroxy-phloridzin to the corresponding *O*-quinone, is maximal at pH 4–5. Biocidal activity of the intermediate oxidation products was particularly strong (RAA, 1968). At high pH phloretin is formed at its highest concentrations, and an *O*-quinone-phloretin coupling reaction takes place. The products of this reaction are reddish brown. The *O*-quinone oxidation product of phloridzin is yellow. These data suggest an explanation for the difference in colour of roots in soil of different pH values, and also of the better growth of apple in acid soils.

Though the role of phloridzin in the etiology of SARD still remains obscure, it is most improbable that phloridzin or its breakdown products do not affect the disease process somehow either to the detriment or to the benefit of re-planted apple trees.

It would not be correct to suggest that phloridzin in apple roots only plays a role in diseased trees; it may very well be that the glucoside is essential for different processes in the healthy tree. One possible aspect is the short term effect on the formation of feeder roots. If it is accepted that phloridzin or its breakdown products are not responsible for SARD, because the persistence of the disease exclude this possibility, it is still possible that the glucoside, or its breakdown products, have short-term effects in the soil which may play a role in the normal process of replacement of feeder roots. BOSSE (1960) showed that feeder roots are often short-lived, and die after an average period of 60 days. New replacement feeder roots are never formed at the site of the former roots. If this short-term effect is based on toxic principles, it could very well be that phloridzin and breakdown products are involved in it.

In some other important specific replant diseases the toxin theory in its 'pure' form. i.e. the disease is directly caused by a toxic substance in the soil, has been suggested, but later abandoned. Such is the case of the 'citrus replant problem' (MARTIN and TSAO, 1968) and the 'peach replant problem'. In the latter, amygdalin was supposed to be able to cause disease (PATRICK, 1955) but later it was shown that the nematode, *P. penetrans* is the most important factor, at least in Canada. Amygdalin nevertheless plays an interesting role in disease etiology and contributes to damage when broken down in the plant as a consequence of the presence of the nematode. Such a relationship could not be demonstrated in the case of *P. penetrans* and phloridzin (PITCHER et al., 1960).

Further work by BÖRNER (1963 a, b, 1965) showed that toxic compounds may be produced by common soil organisms in the decomposition of apple roots, but no indication was found that these compounds were specifically toxic to apple.

5.2.5. *Organisms*

In chapter 4 many cases have been quoted, both of field and pot experiments, in which application of broad-spectrum soil disinfection, by chemicals or heat, leads to normal healthy growth of seedlings or trees planted subsequently in these treated soils. Observation of the root system shows that factors causing a poor condition of the root system in untreated soil have been eliminated by these treatments. These observations are a very strong indication that organisms are involved, because strong biocidal effects are common to all these soil treatments.

Further evidence that organisms are involved in SARD comes mostly by a process of elimination; as shown in the foregoing sections poor soil structure and chemical deficiencies are not causes of SARD, and the direct relation between a toxic residue in the soil and SARD seems most improbable. Most of the other requirements are not strong pointers in favour of the organism hypothesis, but they help in characterizing the responsible organism(s) once these are accepted as the most probable cause.

First the effect of the different treatments tested for controlling the disease will be discussed in some more detail.

Some of the best treatments are chloropicrin, methyl bromide, and heat. These treatments strongly reduce the populations of all major groups of organisms. Viruses are not effectively controlled by many soil fumigants (BROADBENT et al., 1965). There are other indications that viruses can be ruled out as possible causes of SARD. The symptoms do not suggest a virus disease and recovery after transplanting and failure of transmission by grafting (ANONYMOUS, 1960) exclude a virus disease with great certainty.

A number of other soil disinfectants had little or no effect. These observations suggest that it is possible to narrow the range of organisms which could be involved in SARD. Indeed it can be said, for instance in the case of DD, that the limited effects could be related to the fact that certain groups of organisms were not effectively controlled. However, the observed limited effects have to be interpreted with caution (section 2.5).

The small effect of DD is an indication that nematodes are not involved in SARD, but it does not constitute proof. If one supposes that SARD may have a multiple cause, i.e. different factors contributing independently to disease, the elimination of one of these factors does not necessarily lead to a marked growth improvement, if the other factors are still present in a high enough concentration to cause growth reduction. This is especially true in cases of non-lethal organisms, since no disease intensity causing more than 100% growth reduction can be measured. These theoretical considerations should be taken into account when interpreting limited effects on disease of any more or less specific soil treatment.

There are, as already discussed, many other indications which together make it possible to exclude nematodes as factors in SARD.

Methyl isothiocyanate (MIT) and related compounds have failed to control SARD in many cases (sections 4.3 and 4.4.4., SAVORY, 1966, 1967). MIT and related compounds killed nematodes effectively, and also had a good weed-killing effect and, in some preliminary experiments with apple soils, fungi also were very strongly suppressed. The fungicidal effect of MIT is, moreover, generally recognized. As shown in the discussion of the effect of DD further observations are needed before a definite conclusion can be drawn. A second point concerns the organisms which are left over after soil treatment with MIT. According to REBER (1967 a and b) surviving organisms are bacteria and actinomycetes (cf. DOMSCH, 1959b).

The other fungicides, with little or no fumigant action were mostly not very effective, but their range of action is mostly limited. For these reasons it is difficult to interprete the effects of most of the fungicides. An exception is TMTD, which was quite effective in some pot experiments. DOMSCH (1963 b) mentions its effectiviness in killing soil streptomycetes.

Fungi were not generally found associated with diseased roots. *Phycomycetes* were sometimes observed (cf. FRITZSCHE and VOGEL, 1954) and results by MULDER (1968) suggested that damage by species in this group to apple is possible. Although our own observations of the root systems, and the absence of an appreciable effect of MIT, and of DD, which has been found to control *Phytophthora* (ZENTMEYER et al., 1967) and *Pythium* spp. (PARRIS, 1945), as well as the favourable effect of high soil moisture content on growth of apple (cf. McINTOSH, 1964; CONVERSE and SCHWARTZE, 1968), suggest that this group of fungi is not the main cause of SARD, it is certainly possible that they contribute to damage in some cases. Non-lethal attack of feeder roots, leading to reduced growth, has indeed been reported as a symptom in the case of these fungi (HENDRIX et al., 1966; NEWHOOK, 1961). *Pythium ultimum* is considered to play an important role in replant diseases of strawberry (WILHELM, 1965) and peach (HINE, 1961; MILLER et al., 1966). CAMPBELL and HENDRIX (1967) think that *Pythium* and *Phytophthora* spp. may cause disease problems in monocultures of horticultural crops, by attacking the fine absorbing roots. There is no doubt that the study of the relation of *Phycomycetes* to apple roots, may at least contribute to our general knowledge about the reaction of the plant under the conditions of attack by a non-lethal soil-borne pathogen.

If indeed several organisms, whether or not important factors in SARD, could be demonstrated to cause non-lethal growth reduction to apple seedlings, it is strongly suggested that the non-lethality of the disease is rather based on a character of the host plant than of the causal organism.

A root rot of apple in New Zeeland, caused by a Basidiomycete, is clearly different from SARD (TAYLOR and NEWHOOK, 1966).

In all discussions on the causal organisms of SARD, it should be remembered that the organisms responsible may act externally, i.e. be harmful to the root without much direct parasitism. Toxic principles would then be involved, by interaction of products in the rhizosphere, released as exudates or by breakdown of tissues, and the organisms. Apple is known to produce exudates rather abundantly (ROGERS and HEAD, 1966) and from research by PITCHER and FLEGG (1965) and PITCHER (1967) it seems that the exudates are attractive to nematodes *(Trichodorus viruliferus)*: such exudates may favour other organisms as well. The idea that organisms can do harm to plants by the production of toxic compounds from materials present in, or released by the host plant, is far from rare in phytopathology and is certainly a hypothesis which is very different from the simple toxin hypothesis.

The persistence and specificity of SARD suggest that the organisms responsible either form resting stages or have strong saprophytic ability. It is perhaps more probable that the organisms are polyphagous and maintain themselves on other plants without causing much damage to these.

The other requirements in section 5.2.1. mostly do not contradict the organism hypothesis. Perhaps the most surprising result is the absence of a marked effect of soil temperature.

Though high soil moisture content favours both growth in the control pots and in the CP treated soil, the growth of the control plants is stimulated more than the growth of treated plants.

The experiments with mixtures of different proportions of fresh and apple soil may very well be explained by the organism theory. However, the organism involved must be unable to multiply easily in the presence of recently steam-sterilized soil, which is true of many other soil-borne pathogens. An inability to rapidly invade uninfected soil is in agreement with observations which indicate that the causal factor does not move horizontally in the field.

The favourable effect of low soil pH upon the incidence of SARD in apple soils may act by different mechanisms, directly, or indirectly, upon organisms involved. A direct effect on the soil microflora would probably suppress bacteria and actinomycetes, and favour fungi. Secondly there may be an effect of phloridzin breakdown products which will be different at different levels of pH (section 5.2.4.). There may also be interaction with changes in the general condition of the plant caused by lowering the pH, in particular with regard to the nitrogen relationships in the plant and in the soil. Acidification of soil strongly inhibits nitrification (BLASCO and CORNFIELD, 1966); this is also a side-effect of soil fumigation. Growth of apple in both cases shows that apple accepts nitrogen very well in its ammoniacal form. Some plants are known to have a preference for ammonia nitrogen and even do not tolerate nitrate nitrogen because of the absence of a nitrate-reducing system (TOWNSHEND; 1966, TOWNSHEND and BLATT, 1966).

For apple it was shown that blocking of the nitrate-reducing system leads to growth reduction and also that the form in which N is administered is extremely important for the whole process of growth and flower induction (GRASMANIS and NICHOLAS, 1966; GRASMANIS and LEEPER, 1967). TROMP and OVAA (1967) found that the composition of the nitrogen fraction of xylem sap of apple changes through the year and they suggest that a special relation between root and shoot exists with respect to nitrogen metabolism. It is clear that, if such a special relationship exists in the healthy apple tree, the disturbance of the mechanisms which are at the basis of it could be harmful to the tree. Xylem sap of apple does not normally contain nitrate nitrogen (BOLLARD, 1953). The nitrate-reducing system is probably almost exclusively located in the finer rootlets. These are the ones which show decay in the case of SARD. Therefore, as an hypothesis, it may be proposed that the damage observed in SARD is connected with the disturbance of the nitrate-reducing system, and that the beneficial effect of low soil pH may be, at least partially, based on the absence of nitrate.

As a general consideration it seems clear that the processes involved in the etiology of disease, are perhaps related to elementary processes normally occurring with healthy apple trees, and which may be, at least partially, useful for the tree, but which temporarely prevent a normal growth under the unbalanced conditions of replanting. Anyway, in these processes organisms are involved.

It will be clear, that, like nematodes, fungi are probably not the main factor in the causal mechanism, and bacteria and actinomycetes may be more probably involved. The fact that SARD is more prevalent on dryer soils (cf. HILKENBAÜMER, 1964; WAKSMAN, 1967) and also in view of other observations, e.g. limited effect of lower dosages of methyl bromide, less effective against actinomycetes (REBER, 1967 a and b; VAUGHAN et al., 1966), actinomycetes seem to be a group of organisms which deserves special attention.

These final remarks also may suggest lines of future research. It is evident that the cause of SARD is an interesting problem, the further study of which may offer new insights into important aspects of soil biology and their relation to higher plants. It will also be evident that teamwork should highly be recommended, and that the approach to the problem should not only be concentrated on the causal organisms, but also extend to studies on fundamental processes taking place in the healthy and diseased plant.

5.3. Practical aspects

Of the broad spectrum treatments found to be effective against SARD, only chloropicrin has been adopted in practice so far. One advantage of chloropicrin is its reliability in controlling SARD, even under unfavorable soil conditions. This reliability is undoubtedly connected with the rate of application, which is rather high. In England (Savory, 1967) the recommended rate is about 50% of that normally applied in the Netherlands (50 ml/m^2). The treatment is costly, but other treatments are still more expensive. The compound is highly toxic to man, but the gas is very irritating to eyes and throat, thus warning for its presence. This property is extremely useful from the point of view of avoiding accidents. The obligatory plastic sheet used to cover treated soil also helps to prevent accidents and damage to crops on adjoining fields. At the rather high dose of 100 g/m^2 methyl bromide has on average proved to be as effective as CP. The product has the advantage of a very low boiling point (4 °C), and thus waiting periods after application can be very short. This property is, however, not as important for fruit growing in the open as it is in greenhouse crops. Methyl bromide may be useful in cases in which spring application followed by spring planting are desired. Costs of application are somewhat higher than of CP, partly because of the heavier quality of plastic cover needed after application. Methyl bromide is dangerous to man, and odourless; therefore, extreme care should be taken in its application.

Heat treatments are much too expensive to be applied in practice. If, in the future, apparatus should come available in which soil can be heated easily to 60 °C with steam-air mixtures, the possiblity of heat treatments against SARD might again be considered. Thiram was recently found effective in pot experiments, and it should be further tested in the field. Though application might be less expensive than CP treatment, practical problems of mixing thiram, which has no fumigant action, into the soil, should not be underestimated. The product can not be applied at planting time, because it is somewhat phytotoxic.

The pot test is clearly a great help in the practical control of SARD. In cases of severe disease intensity, and in cases of no disease, it is clear which is the advice to be given to the grower. But, especially with light or moderate disease intensity, the profitability of CP treatment still poses problems because of the recovery of untreated trees in the second or third growing season. When more experience is gained with experimental fields, and in general practice, of the performance of older CP treated plantations, the discussion on the profitability, and the interpretation of pot test results, can be made on a firmer basis. The production figures so far available suggest that indeed the development of trees on CP treated plots is such as would normally be expected in good growing orchards (SPOOR, 1967).

The pH effect may be useful in practice, i.e. lowering the soil pH before planting and even before grubbing the old orchard. Also, the favourable effect of high soil moisture content on the growth of replanted seedlings in pots, deserves attention in field experimentation, in combination with soil fumigation. So far insufficient information is available to recommend either sulphur or irrigation for the control of SARD. Irrigation has been successfully applied in the control of *Streptomyces scabies (Actinomycetes)*, the cause of potato scab (LABRUYÈRE, 1965).

It is not recommended to try to avoid the consequences of SARD by planting more vigorous rootstocks than would have been chosen if the same type of plantation were made on fresh or CP treated soil. Such a procedure good give seemingly good results in the first year. But it is clear that the recovery in later years could bring the grower in great difficulties when the trees become too big for the planting system as used for the normally planted dwarfing rootstocks, and also because the trees would lack the other favourable properties of trees on these rootstocks.

6. SUMMARY

In replant diseases of apple, two main factors can be distinguished: damage by nematodes, and specific apple replant disease (SARD), not caused by nematodes.

On light soils nematode damage is prevalent, mostly caused by one species, the endo-parasitic *Pratylenchus penetrans* (Cobb). This nematode is not very harmful in heavier soils (chapter 2).

On the latter soils, serious problems often occur in replanting apple when no appreciable infestation with plant parasitic nematodes is present, which often is the case. In accordance with this is the observed ineffectiveness of nematicides, and, in particular, of DD, in controlling SARD in these cases.

Because fruit growing in the Netherlands is mostly practised on heavier soils, attention was concentrated on SARD, whose causal factor was unknown. The most important points in characterizing the disease are (section 3.1.):

1. Attack confined to feeder roots, disease not-lethal, recovery of trees starting two to three years after planting
2. Causal factor persistent in the soil for several years, not influenced by any crop so far as known, organic soil amendments have little or no effect
3. Specificity to pome fruit, especially affecting apple
4. Quick recovery of trees and seedlings after being transplanted to fresh soil.

The main point of difference between SARD and the disease caused by *Pratylenchus penetrans*, is specificity, which is lacking in the latter case. In the case of SARD there is no obvious cause.

In most fruit growing areas SARD occurred in about 60% of the soils tested; half of these were seriously infested. In some areas the situation is different: In the Bangert area in the province of North Holland, and in the North East Polder in the former Zuiderzee, the disease occurs generally, while in the South of the province of Limburg the disease is not very serious.

In research on SARD, emphasis has been laid on the practical problems for the grower. Secondly, many observations and experiments were directed towards improving insight into the etiology of the disease. In this part of the work experiments were done on the effect of conditions on disease occurrence, disease transmission and, reproduction of symptoms (section 3.3.). These two aspects of the work were integrated as much as possible in the research programme.

The main result of practical interest (sections 4.3. and 4.4.) is that SARD can effectively be controlled by treatments with a broad spectrum of action against soil organisms, i.e. those treatments which strongly reduce the infestation level of all important categories of soil organisms, e.g. nematodes, fungi, bacteria and actinomycetes. Examples of effective treatments are heat (60°C for 30 minutes) and soil application of various chemicals: chloropicrin, methyl bromide and propylene oxide. In pot experiments thiram was also found effective.

As yet, chloropicrin is the only treatment to be applied on a practical scale.

In 4 years experience of this product, it has generally given satisfactory results.

Methyl bromide offers possibilities especially for spring application because it has a lower boiling point and does therefore not demand a long waiting period between fumigation and planting, as in the case of chloropicrin.

Since SARD does not occur in all apple soils, and the treatment with chloropicrin is costly, it is extremely important for the grower to know if a given field is infested. A pot test has been developed (section 4.6.), based on the reproduction of SARD under the conditions of a pot test, and on the correlation between results of CP treatment obtained in the field and in the pot test.

Research on the background of the disease showed that mixing 10% of diseased soil with non-infested soil lead to a marked growth reduction of apple seedlings, but all other attempts to transmit disease or reproduce symptoms failed. These treatments included mixing soils with apple roots and phloridzin, and application of leachates from apple soils. Low pH soils are clearly less heavily infested than near-neutral soils, and acidification of the latter leads to a growth-stimulating effect which is equal to the chloropicrin effect. These observations may offer possibilities for practical application in the future.

When interpreting pH effect in relation to a causal factor different possibilities can be considered: there may be a direct effect on the causal factor, but low pH may also act indirectly, for instance by affecting the nitrogen balance in the soil, i.e. the ratio in which ammonial and nitrate nitrogen are present, which is raised at low pH values by inhibition of nitrification. It may very well be that factors such as nitrogen balance interfere with the etiology of the disease (chapter 5).

The favourable effect of a range of broad-spectrum treatments of the soil in both pot and field experiments, could well be based on the ability of these treatments to control soil bacteria and actinomycetes, because ineffective treatments fail to check these groups of organisms.

In this respect, methyl isothiocyanate and related products are interesting, because they are ineffective in controlling SARD. They control soil fungi and not bacteria and actinomycetes.

In the discussion of the possible causes of SARD, poor soil structure, and chemical deficiencies were ruled out. It seems obvious that soil organisms are an essential factor in the etiology of this disease. Bacteria and actinomycetes deserve special attention because of the selective effect of products as indicated above, the pH effect, and negative indications with regard to fungi. The toxin theory has not been substantiated but it should be stressed that chemicals, including the breakdown products of compounds exuded from the living roots, may very well play a role in the disease process. In such a context, the toxin theory should not be rejected entirely, although toxins are not considered the primary cause.

The need for team work in the continuation of the work has been stressed. It is also clear that more basic knowledge of the general physiology of the apple tree would be very advantageous to future research on SARD.

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[5] now: May and Baker, Ltd.

8. SAMENVATTING

Bij het onderzoek over de herbeplantingsziekten bij appel zijn twee hoofdfactoren naar voren gekomen: schade door aaltjes, en de specifieke herbeplantingsziekte, of specifieke moeheid, die niet door aaltjes veroorzaakt wordt.

Op lichte gronden is aaltjesschade het belangrijkst, voornamelijk veroorzaakt door de in de jonge wortelschors levende soort *Pratylenchus penetrans* (Cobb). Dit aaltje komt ook op zwaardere gronden voor, maar richt daar minder schade aan (hoofdstuk 2).

Op zwaardere gronden treden dikwijls ernstige problemen op bij herbeplanting met appel, ook al zijn geen schadelijke aaltjespopulaties aanwezig, hetgeen vaak het geval is. In overeenstemming hiermee is de waarneming, dat in deze gevallen met specifieke nematiciden, waarvan vooral dichloorpropaandichloorpropeen mengsel (DD) gebruikt werd, een meestal slechts geringe groeiverbetering bereikt wordt. De oorzaak van de slechte groei in deze gevallen is specifieke moeheid.

Omdat de appelteelt in Nederland hoofdzakelijk op zwaardere gronden bedreven wordt, werd aan specifieke moeheid, waarvan de verwekker onbekend is, is, de meeste aandacht besteed. De ziekte wordt als volgt nader gekarakteriseerd (hoofdstuk 3.1.):

1. Aantasting bepaalt zich tot de fijnere wortels; ziekte is niet dodelijk voor zaailing of boom. Bij bomen treedt herstel (doorgroeien) op in het tweede of derde jaar na het planten.
2. De ziekteverwekker blijft minstens 5–10 jaar in de grond achter, niet beïnvloed, voor zover bekend, door enige tussenteelt. Organische toevoegingen aan de grond (stalmest, turf, compost) beïnvloeden het ziekteverloop evenmin.
3. De ziekte is specifiek (vandaar de naam); andere gewassen dan appel groeien goed op voormalige appelpercelen, met uitzondering van peer, die echter minder gevoelig is dan appel.
4. Bij overplanten op verse grond treedt snel herstel op.

Het belangrijkste verschilpunt tussen specifieke moeheid en schade veroorzaakt door *Pratylenchus penetrans* is de specificiteit. Het aaltje is in het geheel niet specifiek voor appel, het tast talrijke andere gewassen aan en is met name schadelijk voor veel soorten houtige gewassen.

In de meeste fruitteeltgebieden komt specifieke moeheid in ongeveer 60% van de gevallen voor; de helft hiervan is zwaar besmet. In enkele gebieden ligt de situatie anders: In de Bangert en in de Noord Oost Polder is de ziekte zeer algemeen, terwijl in Zuid Limburg betrekkelijk weinig schade voorkomt.

In het onderzoek over specifieke moeheid is de nadruk gelegd op de practische aspecten. In de tweede plaats zijn waarnemingen en proeven gedaan met de bedoeling meer inzicht te krijgen in de achtergronden. Bij dit onderdeel van het onderzoek werden proeven gedaan over het effect van de uitwendige omstandigheden op het ziekteverloop, over de overbrenging van de ziekteveroorzaker, en het opwekken van symptomen (hoofdstuk 3.3.).

Het belangrijkste resultaat van practisch belang (hoofstuk 4.3. en 4.4.) is dat specifieke moeheid goed te bestrijden is door behandelingen met een breed spectrum van werking tegen bodemorganismen. Voorbeelden van effectieve behandelingen zijn warmte (60 °C gedurende 30 minuten) en toepassing van verschillende chemicaliën: chloorpicrine, methylbromide en propyleenoxyde. In potproeven werden ook goede resultaten bereikt met thiram.

Alleen chloorpicrine wordt op enige schaal (40 ha per jaar) toegepast. Er is nu vier jaar ervaring mee, en de resultaten zijn in het algemeen gunstig.

Methylbromide biedt mogelijkheden bij voorjaarstoepassing – chloorpicrine wordt in de nazomer of herfst toegepast – omdat het een lager kookpunt heeft dan chloorpicrine, en de wachttijd dus korter kan zijn.

Omdat specifieke moeheid niet in alle appelgronden voorkomt, en de behandeling met chloorpicrine kostbaar is (ongeveer f 1800,– per ha), is het voor de teler belangrijk om te weten of zijn te rooien of gerooide percelen besmet zijn. Ten behoeve van de advisering werd een biologische toetsmethode ontwikkeld gebaseerd op de reproductie van specifieke moeheid in potproeven met zaailingen, en de hierbij gevonden correlatie met de resultaten van veldproeven (hoofdstuk 4.6.).

Bij het onderzoek over de achtergronden van de ziekte bleek dat het mengen van 10% geïnfecteerde grond met verse of gestoomde grond tot een duidelijke groeiremming leidt, maar andere manieren waarop geprobeerd werd de ziekte over te brengen of te reproduceren, leverden geen resultaat op. Tot deze behandelingen behoorden het mengen van grond met appelwortels en met phloridzine (een glucoside uit de appelwortel), en het toevoegen van percolaten van appelgrond aan niet geïnfecteerde grond. Gronden met lage pH zijn minder sterk besmet dan de ongeveer neutrale gronden. Bovendien kan door kunstmatige pH verlaging van besmette grond een even sterke groeiverbetering worden bereikt als met chloorpicrine. De mogelijkheden voor toepassing van deze waarnemingen voor de praktijk zijn nog in onderzoek.

Het gunstige effect van een lage pH kan berusten op een rechtstreekse invloed op de ziekteverwekker, maar er kan ook een indirecte invloed zijn b.v. via de verhouding tussen de ammoniakale en nitraatstikstof in de grond, die door een lage pH sterk verhoogd wordt. Als hypothese is gesteld dat een storing van het nitraatreducerend mechanisme in de wortels verband kan houden met de ziekte (hoofdstuk 5).

De effecten van de toegepaste bestrijdingsmethoden wijzen uit dat organismen van essentieel belang zijn in het ziekteproces. Zoals het DD effect aaltjes uitsluit, kan het geringe effect van methylisothiocyanaat (MIT) geïnterpreteerd worden als een aanwijzing dat ook schimmels niet de hoofdoorzaak zijn, omdat MIT een sterke fungicide werking heeft. Bacteriën en actinomyceten overleven de behandeling met MIT, en omdat de ziekte vooral in drogere grond sterk optreedt, zou in het toekomstig onderzoek ook vooral aan actinomyceten aandacht besteed moeten worden. Het pH effect wijst er ook op dat bacteriën en actinomyceten eerder als ziekteverwekkers in aanmerking komen dan schimmels.

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