AGRONOMIC STUDIES ON THE POPULATION DYNAMICS OF *VERTICILLIUM DAHLIAE*

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AGRONOMIC STUDIES ON THE POPULATION DYNAMICS OF *VERTICILLIUM DAHLIAE*

PROEFSCHRIFT

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 23 juni 1995
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STELLINGEN

1. Terugdringing van het gebruik van grondontsmettingsmiddelen leidt tot een toename van de schade door *Verticillium dahliae* in het gewas aardappel.

2. Wortels van cultuurgewassen stimuleren de kieming van microsclerotia van *V. dahliae* in de bodem, maar dit proces heeft voor de beheersing van de ziekte nauwelijks betekenis.
   Dit proefschrift

3. De virulentie van een *Verticillium dahliae* populatie in de bodem hangt af van de teeltgeschiedenis van het perceel en het te telen gewas.
   Dit proefschrift

4. De reproduktie van *V. dahliae* is, onder gunstige weersomstandigheden, lager bij mechanische loofdodingstechnieken dan met chemische loofdoding.
   Dit proefschrift

5. Bij de veldkeuring van pootaardappelen is visuele beoordeling op aantasting door *Verticillium dahliae* onmogelijk, onnodig en elk resultaat misleidend.

6. Bij de Landbouwuniversiteit hoort de boer de hoogste troef te zijn.

7. Regelgeving door de overheid met betrekking tot de landbouw wordt beïnvloed door zoveel partijen die een te geringe kennis van de praktijk hebben dat regels vaak tot te hoge kosten voor de ondernemers leiden en te weinig doeltreffend blijken.

8. Door een gebrek aan specifieke kennis bij milieu- en natuurbeschermingsorganisaties heeft veel kritiek op boeren en jagers een emotionele basis gekregen. Door bij propaganda ook op de emoties van het publiek in te spelen wordt bewust de kloof tussen opvattingen van de partijen vergroot.

9. Alleen grondgebonden produktie van plantaardige en dierlijke produkten behoort landbouw genoemd te worden. Als deze definitie wordt aanvaard, wordt de landbouw meteen een stuk milieuvriendelijker.

10. Flexibilisering van de werktijden van chauffeurs in het streekvervoer kan eenvoudig en zonder extra kosten worden ingevoerd door de onregelmatigheidstoeslagen aan te passen en elke chauffeur individueel deze toeslag uit te betalen. (Dit laatste in tegenstelling tot de bij sommige bedrijven nu gangbare gemiddelde onregelmatigheidstoeslag.)

11. Een onmisbare vaardigheid van de promovendus is dat hij het handschrift van de promotor goed en snel kan ontcijferen.

12. Door de ontwikkeling van een goede methode om uien panklaar aan te bieden kunnen veel tranen in de keuken worden voorkomen.

13. Mollen graven dieper dan menigeen denkt.

14. Van het kunnen is slechts één bewijs: het doen!

Stellingen behorende bij het proefschrift:

"AGRONOMIC STUDIES ON THE POPULATION DYNAMICS OF *VERTICILLIUM DAHLIAE*"

Leon Mol

Wageningen, 23 juni 1995

ABSTRACT

Mol, L., 1995. Agronomic studies on the population dynamics of *Verticillium dahliae*. Doctoral thesis, Wageningen Agricultural University, Wageningen, The Netherlands, X + 159 pp., English and Dutch summaries.

Verticillium dahliae reduces crop yields by causing early senescence. The fungus survives in the soil as microsclerotia formed on senescing tissue of colonised plant parts. *V. dahliae* is endemic in many soils because of the high potential survival of its microsclerotia and its wide host range. The primary aim of the research was to investigate the feasability of reducing the inoculum density in the soil by stimulating the microsclerotia to germinate. A second aim was to quantify the formation of microsclerotia on various crop species and cultivars, and various parts of potato plants. Haulm treatments to control the formation of microsclerotia on potato were tested and finally a theoretical model describing the long-term dynamics of the inoculum density in the soil was developed and tested.

All crops investigated stimulated the germination of microsclerotia in the soil. Host plants such as potato and field bean induced more microsclerotia to germinate than a non-host such as barley, but none of the crops was able to reduce the soil inoculum density effectively.

The highest total microsclerotial yield occurred in flax, followed by potato cultivars; the other crops lagged far behind. In potato, the mature aerial parts had the highest numbers of microsclerotia.

Potato cv. Element and field bean proved to be most sensitive to their own isolate. In plots cropped with good hosts, soil inoculum density increased very rapidly. Removing the debris of potato, field bean and flax from the field was effective in reducing the increase of the inoculum density. Mechanical haulm treatments reduced the formation of microsclerotia more than a chemical treatment.

A theoretical model built on the basis of biological and ecological principles gave a very high correlation when it was fitted to the data obtained in a long-term field experiment.

This study provides a quantitative basis on the interactions between crops and *V. dahliae* that deserves to be expanded in further research.

Keywords: crop rotation, crop species, crop debris, cultivars, haulm killing, *Hordeum vulgare*, image analysis, inoculum density, *Linum usitatissimum*, microsclerotia, modelling, population dynamics, potato, removal, reproduction, resistance, root density, root exudates, root observation, soil infestation, *Solanum tuberosum*, tolerance, *Verticillium dahliae*, *Vicia faba*, yield

Voor mijn vader

WOORD VOORAF

De milieuvriendelijke beheersing van ziekten en plagen in de landbouw is niet nieuw. Al sinds meer dan 100 jaar wordt getracht door middel van teeltmaatregelen schade door pathogenen te beperken. Vruchtwisseling speelt hierbij een prominente rol. De vakgroep Agronomie van de Landbouwuniversiteit heeft al een lange en "duurzame" traditie op het gebied van vruchtwisselingsonderzoek. Sinds jaren wordt hieraan vormgegeven door dr ing. K. Scholte. Er mag mijns inziens dan ook gesproken worden van de "School Scholte". Het is niet vreemd dat in het kader van het additioneel onderzoeksprogramma van het Meerjarenplan Gewasbescherming ook teeltkundig onderzoek is opgenomen. Immers, de agronomie is de discipline bij uitstek om basiskennis uit verschillende deelgebieden op een zo hoog aggratieniveau te integreren dat het verkregen eindprodukt een grote maatschappelijke relevantie krijgt.

Het uitvoeren van een promotieonderzoek is niet het werk van de promovendus alleen. Zonder de hulp van vele personen zou dit proefschrift nooit tot stand zijn gekomen. Ik dank hen allen hartelijk hiervoor! Zonder anderen tekort te doen, wil ik een aantal mensen met name noemen.

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Blokzijl en Herry Theunissen dank ik voor het klaren van de vele technische klussen. Gerrit Besselink en Guido van Hasselt dank ik voor het tellen van vele duizenden microsclerotia.

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Mevr. J. Burrough-Boenish corrigeerde op nauwgezette wijze de Engelse tekst van een aantal hoofdstukken.

Ik bedank de VSB-bank, het NWO en het Johanna Westerdijkfonds voor het subsidiëren van buitenlandse reizen naar de Verenigde Staten en Israël in 1992, 1993 en 1994. Het Hilbrands Laboratorium voor Bodemziekten te Assen en het PAGV te Lelystad dank ik voor het beschikbaar stellen van grond voor het verkrijgen van gewaspecifieke isolaten van *V. dahliae*.

Ik draag dit proefschrift op aan mijn vader. Hij heeft me betrokken bij de groei en de teelt van gewassen en me geleerd als boer naar gewassen te kijken. Ik heb dit de afgelopen jaren als onmisbaar ervaren.

Leon

NOTE

All papers included in this thesis are submitted to or accepted by various international journals. The name of the journal concerned is printed at the top of the first full page of each chapter or section. If possible, reference to the contents of the papers should be made by citing the original publications. As presented in this thesis they differ from the original papers in the following ways:

1. the 'keywords' of the individual papers have been combined into one list at the end of the 'Abstract';

2. the acknowledgements are given in the 'Woord vooraf';

3. the 'References' of the general introduction, the different papers and the general discussion have been combined into one list;

4. some minor changes were made to standardize presentation.

I thank Kluwer Academic Publishers (Section 1.2, Chapters 2 & 3), Blackwell's (Chapters 5, 6 & 8) and the editorial boards of Potato Research (Section 4.2; Chapter 7) and the Netherlands Journal of Agricultural Science (Section 4.1) for their kind permission to include the papers in this thesis.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

SECTION 1.1

INTRODUCTION

Verticillium dahliae Kleb. is a serious pathogen affecting potato (*Solanum tuberosum* L.) in most countries where this crop is grown. The fungus survives in the soil by microsclerotia that persist for many years. The density of microsclerotia of *V. dahliae* in the soil mainly depends on the cropping history, since the primary source of the inoculum is infested plant debris. Plant roots can be colonised if microsclerotia germinate in the vicinity of the root tip (Fitzell et al., 1980). Colonisation is followed by systemic infection of the vascular system of the plant, whereafter *V. dahliae* is dispersed within the host by conidia and mycelial growth. Systemic infection by *V. dahliae* affects plant growth and depresses plant yield. Yield reduction is mainly caused by closure of the stomata and early senescence of the canopy after blockage of the vascular system of the haulm (Bowden et al., 1990; Haverkort et al., 1990). Microsclerotia are formed abundantly in infested tissue upon death of the host plant. Since *V. dahliae* has a very broad host range, the influence on soil infestation of all crops in the rotation must be considered. *Verticillium dahliae* shows synergistic interactions with other pathogens, such as endoparasitic nematodes: *Globodera* spp., *Pratylenchus* spp. and *Meloidogyne* spp., and the fungi: *Colletotrichum coccodes*, *Fusarium* spp. and *Rhizoctonia solani* (Riedel & Rowe, 1985; Evans, 1987; Scholte, 1989; Scholte & s'Jacob, 1989). Thus, it may be expected that a reduction in the use of nematicides will indirectly increase the damage caused by *V. dahliae*.

A lower cropping frequency of potato may result in lower densities of *V. dahliae* in the soil, but is not an attractive control method to the farmer, because in The Netherlands potato is a major cash crop. Therefore, other methods to control the inoculum build-up in the soil deserve to be investigated. Since in Dutch crop rotations potato is the crop that suffers most from *V. dahliae* and since the pathogen can form a large number of microsclerotia on potato tissue, the project described in this thesis is focused on this crop.

A wealth of literature concerning life cycle and ecology of *V. dahliae* is available. A short review is given in Section 1.2. The aim of the research is described in Section 1.3, after which the structure of the thesis is given (Section 1.4).

In: Haverkort, A.J. & D.K.L. MacKerron (eds). Ecology and modelling of potato crops under conditions limiting growth. Kluwer Scientific Publishers, Dordrecht, The Netherlands: 251-263.

SECTION 1.2

LIFE CYCLE AND ECOLOGY OF *VERTICILLIUM DAHLIAE* IN POTATO

L. Mol and A.J. Termorshuizen

Summary

Verticillium dahliae is a serious pathogen in most countries where potato is grown. The density of microsclerotia of *V. dahliae* in soil mainly depends on the cropping history. Plant roots can be colonised if microsclerotia germinate in the vicinity of the root tip. Colonisation is followed by systemic infection of the vascular system of the plant. During colonisation of the root cortex and systemic infection of the plant there are interactions with many soil organisms. Cultural practices can lessen the colonisation of the roots and the severity of the disease.

In the vascular system of the plant, *V. dahliae* is dispersed by conidia and mycelial growth. Wilting symptoms appear after the reactions of the plant to the presence of the pathogen. Yield reduction is mainly caused by closure of the stomata and early senescence of the canopy after blockage of the vascular system of the haulm.

The fungus forms microsclerotia on dead plant tissue. External climatological factors and haulm killing practices have a large influence on the number of microsclerotia formed per unit haulm material. Since *V. dahliae* has a very broad host range, attention should be paid to the control of this pathogen in all crops in a rotation. In some crops host specificity has been found, but this is a gradual property.

Introduction

Diseases caused by *Verticillium dahliae* Kleb. are wide-spread throughout the world, wherever susceptible crops are grown, and are of economic importance in most countries (Pegg, 1984). *Verticillium dahliae* is supposed to be the major component of the potato early dying complex that damages crops by causing early maturity of the crop by wilting. Hosts include all dicotylodonous plants, with the most important crops affected being potato, cotton, egg-plant,

tomato, mint, and olive. *Verticillium dahliae* survives by means of microsclerotia (MS). The high survival potential of MS and the wide host range make *V. dahliae* endemic to many agricultural soils (Powelson, 1970).

Verticillium dahliae is classified as a soil invading or root inhabiting fungus (Powelson, 1970). These fungi are characterised by a parasitic phase on the living host plant (Fig. 1: I), and by a saprophytic phase after the death of the host (Powelson, 1970) (Fig. 1: II). The two phases will be discussed separately.

Dynamics of the soil population of *V. dahliae*

A schematic representation of processes concerning the MS population in the soil is shown in Fig. 1: II. The population of MS in soil varies in composition and density depending on the cropping history of the field. After germination of a microsclerotium, the hypha may infect a plant root. Due to their low competitive saprophytic ability, the majority of the hyphae are not successful in reaching a plant root and they die. The next paragraphs will be focused on: survival of MS (Fig. 1: a), germination of MS (Fig. 1: b), and colonisation of plant roots (Fig. 1: c).

Survival of MS (Fig.1: a)

Ben-Yephet and Szmulewich (1985) reported that MS of *V. dahliae* survive longer in the field than in the laboratory. After 5 years of storage of air-dried soil samples at 20-25°C no *V. dahliae* could be detected, whereas 4% of the original population density remained viable after 7 years of crop rotation. Wilhelm (1955) found that *V. dahliae* persisted for 14 years in field soil with no hosts present. The long persistence of the fungus in the field is probably due to its ability to colonise and produce new MS on the root systems of nearly all plant species including monocots (Martinson & Horner, 1962). The density of MS in soil was monitored by Itoh et al. (1989) following Chinese cabbage by using infested soil. A first order rate equation was fitted to the observed decrease in the number of MS which was more rapid at higher temperatures. A linear relation was observed between the logarithmic value of the half life and the temperature in the range of 5 - 31°C.

Survival of MS of *V. dahliae* appears to be best under air-dry conditions (Coley-Smith & Cooke, 1971). The mechanism for survival has not been elucidated. There is a clear relation between the presence of melanin in organisms and their persistence (Bloomfield & Alexander, 1967). Temperature may exert an indirect influence on survival through direct effects on

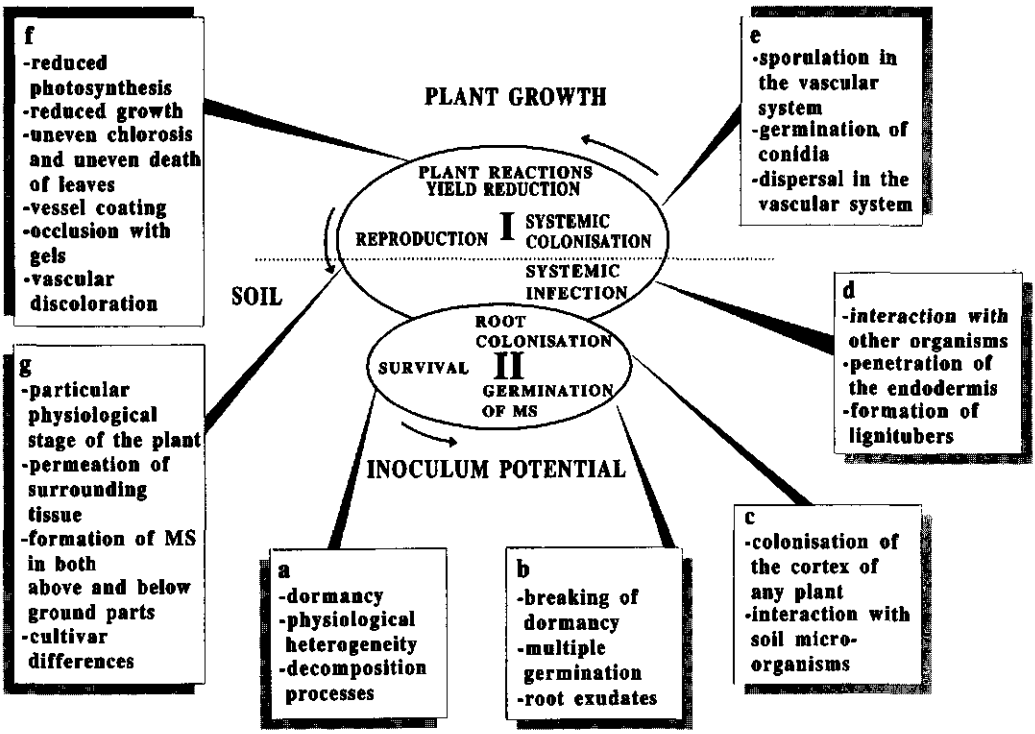

Fig. 1. Schematic life cycle of *V. dahliae* showing a parasitic phase on a host (I), and a saprophytic phase after plant death and in soil (II). The scheme is explained in the text. MS = microsclerotia.

germination of MS (Coley-Smith & Cooke, 1971) and decomposition of the plant material they are embedded in to achieve their release into the soil (Hancock & Benham, 1980). The influences of the temperature and decomposition are entangled. Decomposition is the result of more than one process, and an increase in temperature, within a certain range, will accelerate most biological processes.

The release of MS from colonised plant debris lasts at least one year following its incorporation into the soil and consequently affects the inoculum density in soil. In two cultivated cotton-field soils studied by Evans et al. (1967), the density of MS in soil declined throughout the growing season, but increased again at harvest time when mechanical damage to colonised cotton plants caused the release of fresh MS. There was a further increase in the soil inoculum density when the dead cotton stalks were returned to the soil in preparation for the next crop. Huisman and Ashworth (1976) observed the soil inoculum density of *V. dahliae* in eight commercial fields with different cropping histories at about monthly intervals for 3.5-4.0

years. High inoculum densities in soil persisted under continuous cultivation of cotton. The inoculum density usually increased rapidly following one year of cotton, with a higher inoculum density occurring in the second year regardless of the susceptibility of the subsequent crop. The slow release of MS from decomposing plant debris probably causes a delay in the build-up of the inoculum density in soil. It is not known how the survival of MS embedded in plant material compares to the survival of released 'free' MS. Presumably, the death rate is higher in bound MS because of the higher microbial activity in and around the decomposing plant debris.

Germination of microsclerotia (Fig. 1: b)

Microsclerotia of *V. dahliae* have no constitutive dormancy where development is delayed because of an endogenous property of the propagule (Pegg, 1974), but they do have a requirement during germination for exogenous nutrients (Emmatty & Green, 1969) from root exudates of either hosts or non-hosts (Schreiber & Green, 1963).

Concentrations of exogenous nutrients can be influenced by organic amendments to the soil, which influence general soil microbial activity and concentration of soluble compounds in the soil solution. Increased soil microbial activity has been reported to reduce the proportion of germination of microsclerotia and growth of germ-tubes of *V. dahliae* in the rhizosphere (Jordan et al., 1972). In greenhouse and field experiments, amendments with chopped barley or oat straw at several rates reduced the inoculum density of *V. dahliae* in soil and disease incidence in potato plants (Tolmsoff & Young, 1959; Harrison, 1976). The effect of organic amendments has not been elucidated, but it has been suggested to be an increase in production of antifungal substances by the enriched microflora of the rhizosphere (Curl & Truelove, 1986). However, the interactions between the pathogen and the microflora in the rhizosphere are probably more important, since there is still doubt that antibiotic substances produced in soil can endure microbial degradation for long enough to act as effective inhibitors (Curl & Truelove, 1986).

Microsclerotia have the ability to germinate more than once. Farley et al. (1971) showed that MS germinated and sporulated every time after re-moistening soil with a sucrose solution or water up to nine times. Germination percentage and the number of germ-tubes decreased with succeeding germinations. Where a susceptible crop is planted in a field in soil naturally infested with *V. dahliae*, the behaviour of the MS is likely to be different from that in experiments where artificial inoculum is applied (Menzies & Griebel, 1967). Unless several years have elapsed since the last susceptible crop was grown, the inoculum may be mostly in the form of MS embedded in partly decomposed plant residues. Some of these MS may already have undergone a cycle of germination and be depleted, while others may be freshly exposed during

cultivation operations and may go through a peak of germination as the host seedling roots are growing in their vicinity (Menzies & Griebel, 1967).

Colonisation of plant roots by V. dahliae (Fig. 1: c)

Germination hyphae of MS may infect susceptible hosts by penetration of the root cortex followed by systemic invasion of the xylem vessels (Powelson, 1970).

Infectious hyphae of *V. dahliae* emerging from MS penetrate roots primarily near the root tip and in the root-hair zone (Fitzell et al., 1980; Gerik & Huisman, 1988). The density of colonisation at a distance of more than 1 cm from the root apex appeared to be constant (Gerik & Huisman, 1988). Most of the colonies are removed when the roots are surface-sterilised, indicating that *V. dahliae* is predominantly restricted to superficial sites in the root cortex (Evans & Gleeson, 1973).

Host-pathogen interactions

A schematic representation of the host-pathogen interactions is shown in Fig. 1: I. After colonisation of the plant root, the fungus crosses physical barriers in the plant to reach the vascular system. During systemic infection, colonisation of the plant may result in resistance reactions, yield reduction, and, finally, production of MS on the plant debris. The next paragraphs will be focused on systemic infection of plant roots (Fig. 1: d), systemic colonisation of the plant (Fig. 1: e), plant reactions and yield reductions (Fig. 1: f), and reproduction of *V. dahliae* (Fig.1: g).

Systemic infection of plant roots (Fig. 1: d)

In response to the initial invasion by the fungus, the inner tangential wall of the epidermis becomes swollen (Bell, 1973). Occasionally a gum-like encapsulation zone may be present in the outer cortical cells of the plant roots around the hyphae of *V. dahliae*; these formations are referred to as lignitubers (Griffiths, 1973). Lignitubers result from the extrusion of vesicles from the protoplast into the area between the cell wall and plasmalemma. When the fungus starts to penetrate the cell wall, the vesicles aggregate and lose their individual identity as they form the lignituber. Subsequently, the fungal cell wall becomes surrounded by the lignituber, while the host plasmalemma apparently remains intact. In most cases the invading hyphae lyse, but in a few cases successful penetration occurs and the fungus proceeds through the layer of cortical cells (Bell, 1973).

Fungi that successfully penetrate the cortex encounter a second barrier at the endodermis (Bell, 1973). Most of the penetrating hyphae that were not stopped by lignitubers are so at the endodermis; the few hyphae that penetrate this barrier progress into the vascular tissue and invade the vessels by penetrating through the pits. Alternatively, hyphae may reach the vascular system by colonising the young root tip, where the endodermis still has to be formed. The systemic infection of the plant root is the property determining the suitability of the plant as a host. For example, susceptible mint (*Mentha* spp.) roots showed more extensive systemic invasion, while the number of root cortex infections was similar to that of resistant mint species (Lacy & Horner, 1966).

Infection of plant roots by *V. dahliae* may be influenced by other organisms. In most cases, potato early dying can be attributed to a complex of soil microorganisms including *V. dahliae*, *Globodera* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Colletotrichum coccodes*, *Fusarium* spp. and *Rhizoctonia solani* (Scholte, 1989; Scholte and s'Jacob, 1989). Other pathogenic agents that have been associated with potato early dying include *Erwinia carotovora* and Potato Virus X (Rouse, 1985). It has been established that potato early dying is frequently caused by *V. dahliae* in combination with endoparasitic nematodes. In microplot studies on potato cv. Russet Burbank, Kotcon et al. (1985) did not find a significant contribution by either *C. coccodes* nor *R. solani* to the disease syndrome either alone or in combination with *V. dahliae* or *P. penetrans*. However, in potato cv. Amethyst yield loss by *V. dahliae* was almost doubled in the presence of *C. coccodes* (Scholte et al., 1985), and Scholte and s'Jacob (1989) found a three-factor interaction on yield loss between *Meloidogyne* spp. or *P. neglectus* and *R. solani* and *V. dahliae* with several potato cultivars.

The mechanism of the interaction between *V. dahliae* and *P. penetrans* that results in the synergistic expression of symptoms and yield loss has been subject of speculation. Green (1981) considered that the mechanism could be wounding from nematode feeding, which gives the fungus easier access to the vascular system. Alternatively he proposed a physiological mechanism where the plant becomes more susceptible to the fungus because of a translocated substance. Wheeler et al. (1992) compared a model in which yield loss of potato is proportional to both *V. dahliae* and *P. penetrans* with a model in which yield loss is proportional only to the population density of *V. dahliae* and the presence of *P. penetrans* leads to a more severe yield loss function than in the absence of the nematode. However, their comparison was regarded not conclusive considering the variability in the expression of potato early dying and confounding effects of environmental conditions.

In evaluating his mechanistic model, Johnson (1992) described potato crop losses caused by multiple biotic stress factors, excluding nematodes. He concluded that crop loss by multiple

stress factors was less than the sum of losses from each stress factor acting alone. This was illustrated by a competitive defoliation between *V. dahliae* and *Phytophthora infestans*.

Systemic colonisation of the plant (Fig. 1: e)

The process of production of conidia in the vascular system is still unclear. A hypothesis put forward by several authors is that directly after *V. dahliae* has penetrated the vascular system, it starts to produce conidia (Howell, 1973; Schnathorst, 1981). Conidia are produced by simple conidiophores or by budding (Tolmsoff, 1973) and are passively distributed through the vascular system throughout the plant. Conidia may germinate, and penetrate vessel walls (Garber, 1973; Tolmsoff, 1973). More research concerning the process of sporulation and distribution in the vascular system is needed. For example the influence of the nutrient concentration in the xylem and the influence of the vitality of the plant on these two processes are indicated as origins of differences in the number of *V. dahliae* particles in the stems of plants, but the effect has never been proven in experiments.

In susceptible and tolerant cotton cultivars, colonisation was equal up to the point where the pathogen passed the endodermis and reached the xylem (Garber, 1973). The number of vessels invaded appears to be a good measure of the severity of wilt disease, because the number of invaded vessels was related to the number of systemic infections and to the number of hyphae that progressed through the cortex from the points of colonisation.

As long as petioles of potato plants are green, distribution of *V. dahliae* in the plant is limited to the xylem, but in plants with severe disease symptoms the pith, cambium, and cortex are invaded (Garber, 1973). In the leaves, infection may be confined to a single pinna, or may involve the entire leaf (Garber, 1973).

Plant reactions and yield reductions (Fig. 1: f)

In potato, symptoms of *V. dahliae* are difficult to distinguish from normal senescence and may initially involve only reduced growth (Street and Cooper, 1984; Haverkort et al., 1990). Early foliar symptoms may appear as unilateral chlorosis of lower leaves on a few plants. Later some wilting of whole leaflets or leaves may occur, but the unilateral death of lower leaves is more typical (Isaac & Harrison, 1968).

The activities of the fungus stimulate the plant to produce a suberin-like coating, tyloses and gels inside the vascular elements. Tyloses are extensions from parenchyma cells into the xylem (Newcombe & Robb, 1988). Gels arise from perforation plates, end-walls and pit membranes by a process of distension of primary wall and middle lamella constituents (Molen et al., 1977). This can lead to occlusion of the vessels in the vascular system immediately above primary

infection sites (Molen et al., 1977; Harrison & Beckman, 1982; Newcombe & Robb, 1988). A light brown vascular discolouration is often visible at the stem base when sliced (Isaac & Harrison, 1968). The accumulation of specific chemicals such as terpenoid aldehydes in the vessels accompanies or follows the presence of the pathogen and reduces the viability of the fungal propagules (Harrison & Beckman, 1982). As colonisation of the xylem proceeds, the vessels may become plugged by hyphae (Garber, 1973). In potato plants the plugging has been traced from the root tip up to the top of the stem.

Inoculated potato plants produced no symptoms until tuberisation commenced (Busch & Edgington, 1967), suggesting that before symptom expression of Verticillium wilt becomes evident, the host must be in an advanced stage of development (Busch et al., 1978). By altering the photoperiod to prevent tuberisation, few or no symptoms developed (Busch & Edgington, 1967). These observations are consistent with other observations of an association between lateness of cultivar and resistance to *V. dahliae* (Busch et al., 1978).

Kotcon et al. (1985) associated disease incidence of *V. dahliae* with reduced root growth, foliar weight, and tuber yield. Infected plants exhibited lower specific leaf areas (area produced/dry weight of leaf tissue), higher leaf weight ratios (dry weight of the leaf system/dry weight of the whole plant) and higher leaf area ratios (area of the leaves produced/dry weight of the whole plant), and, under dry conditions, lower relative growth rates and lower leaf growth rates (increase in dry weight/unit leaf area/week) (Harrison & Isaac, 1969).

Bowden et al. (1990) and Haverkort et al. (1990) showed that the initial decrease in photosynthesis caused by *V. dahliae* was caused by stomatal closure. The low stomatal conductance was correlated with low leaf water potential (Bowden et al., 1990). In potato, *V. dahliae* caused a reduction in the light conversion efficiency, but a stronger reduction of stomatal conductance, resulting in decreased internal/external CO_2 ratios and in a higher net photosynthesis at similar values of stomatal conductance (Haverkort et al., 1990). The reduction of net photosynthesis does not seem to be responsible for more than 10% of the reduction of dry matter production. In areas where the disease causes an early and rapid senescence of the leaf canopy, reduction of intercepted radiation may be a more important component of damage than reduction of photosynthesis (Haverkort et al., 1990).

Although there are interconnections between the vascular bundles in the stem, these are absent in the petiole. As a consequence blockage of petiole bundles can be more damaging than a proportional blockage of stem bundles (Garber, 1973).

A lesser root length of potato plants due to *V. dahliae* may decrease the water supply and cause the development of foliar symptoms (Kotcon et al., 1984). Also root surface areas and volumes were reported to be affected negatively by *V. dahliae*.

Reproduction of V. dahliae (Fig. 1: g)

As infected plants become senescent, the fungus permeates the surrounding tissues and forms MS within dead tissue (Powelson, 1970). There is no evidence of other than transient increases in inoculum from sources other than plant debris.

Potato stems colonised by *V. dahliae* will be filled with MS. A 1 cm segment of stem may contain 8,000-20,000 viable MS and populations up to 1,000 MS per gram of soil have been reported in fields repeatedly cropped to potato, which roughly equals 50 million MS in the soil volume occupied by the roots of one plant (Menzies, 1970). Differences in production of MS from 7,000-9,000 propagules per gram of stem tissue have been reported in several potato cultivars (Slattery, 1981).

In most plant species infected with *V. dahliae*, water is required for formation of MS (Powelson, 1970). However, in the temperate zones, humidity is usually sufficiently high during the senescence of the potato haulms to ensure abundant MS formation without rain.

External factors can have a large influence on the formation of microsclerotia. Autumn-grown crops of potato in the Negev area of Israel had approximately 100 times higher microsclerotial production than the spring-grown crops (Ben-Yephet & Szmulewich, 1985). Either the cool and moist conditions in the autumn-winter season enabled the plants to dry slowly, favouring formation of MS, or the cooler weather allowed better survival of the fungus in the plant tissue.

Ioannou et al. (1977b) examined the formation of MS in tomato debris in soil subjected to different irrigation and flooding regimes under field conditions. Few, if any MS were produced during the flooding treatment. This inhibition was attributed to decreased O_2 and increased CO_2 concentrations in the flooded soil. Upon drainage, the concentrations of O_2 and CO_2 returned rapidly to normal atmospheric levels and formation of MS was resumed. The numbers of MS eventually produced following 10-, 20-, and 40-day flooding treatments were 90, 44, and 46% respectively, of the average numbers in the non-flooded treatments.

The major part of the new inoculum is produced in the aerial parts of the potato plant (Ben-Yephet & Szmulewich, 1985). A direct way for control of *V. dahliae* is to interfere with the formation and dispersal of MS. In practice this is accomplished by various sanitation measures such as removal or destruction of diseased plant material before it enters the soil or before it releases the inoculum otherwise. This process of field sanitation is not widely practised because of expense, lack of equipment, and through farmers' aversion to destroying organic matter.

Production of MS has been reported in plant roots without systemic infection. Microsclerotia were formed in large numbers in only few sections of wheat roots (Krikun & Bernier, 1990). Taking into account total root length of wheat and the number of MS found (up to 100 per root

fragment of 1.3x0.4 mm), the contribution to the inoculum in soil may be significant (Krikun & Bernier, 1990).

Weed control is important to limit multiplication of *V. dahliae* (Woolliams, 1966; Johnson et al., 1980). Host species include many common weeds and native plants. As in crop plants, symptoms are not always apparent in infected weed plants.

Host specificity

Adaptation of *V. dahliae*, leading to host specificity, may confuse the search for possible resistance or tolerance mechanisms. Zilberstein et al. (1983b) reported that germination on agar and pathogenicity of MS of *V. dahliae* to egg-plant, potato and tomato was affected by growth medium and host origin. The virulence of an isolate of *V. dahliae* depends on the host species, and geographical origin, susceptibility of the host cultivar/genotype, and the organ on the plant (Zilberstein et al., 1983b; Michail, 1989). Isolates obtained from susceptible cultivars attacked these cultivars only. Isolates from resistant cultivars attacked both susceptible and moderately resistant cultivars (Michail, 1989).

Production of MS variants might permit the fungus to adapt to new host species and varieties or to new environmental conditions (Tolmsoff, 1973). Mint isolates were originally not pathogenic to tomato. After one passage through tomato, however, the isolates became more pathogenic to tomato and lost pathogenicity to mint. The isolates from a region of continuous cropping to one crop tend to be similar and all display high virulence against that particular crop but generally a weak virulence against other species which they can infect nevertheless (Vigoroux, 1971). Thus a notion of "preferential" and "occasional" hosts is formed. It is known that great variability is possible in *Verticillium* species (Vigoroux, 1971).

Puhalla and Hummel (1983) found 16 different vegetative compatibility groups in *V. dahliae*. Using another method, Joaquim and Rowe (1990, 1991) were able to reduce the number of vegetative compatibility groups to four. Isolates from two different vegetative compatibility groups showed significant differences in pathogenicity towards potato (Joaquim & Rowe, 1991).

Thus, in soils, populations of individuals with different properties can be built up. It appears that the kind of crop constitutes a determinant factor for the quantitative and qualitative composition of the population of *V. dahliae* in cultivated soils (Vigoroux, 1971; Tjamos, 1981). So, the choice of cultivar, crop rotation, and cultural practices will not be sufficient to keep the crop from infection, but they will still be key factors in the controlling the severity of the disease.

SECTION 1.3

AIM OF THE RESEARCH

In this thesis, various processes in the life cycle of *V. dahliae* are analysed quantitatively to arrive at a holistic analysis of a potential control strategy. Quantitative information about formation and mortality of microsclerotia of *V. dahliae* is poor and not consistent. Since both hosts and non-hosts can induce germination of microsclerotia (Schreiber & Green, 1963), a possible strategy to control *V. dahliae* might be to grow non-hosts. Indeed, inducing microsclerotia to germinate, without producing abundant propagules after plants have been infected, may be a major factor in the control of Verticillium wilt when non-hosts are used in rotations (Schnathorst, 1981). The effect of the roots of commonly grown field crops on microsclerotia is not known, but needs to be quantified before the search for a special 'luring' crop can be started.

Formation of microsclerotia occurs in large numbers on hosts. Also on non-host crops *V. dahliae* may form microsclerotia on the roots without systemically infecting the plant. This limits the possibilities to reduce the population by induction of the germination of microsclerotia.

The most direct way of controlling *V. dahliae* is to limit the formation and dispersion of new inoculum. Potato haulm treatments in the field or removal of crop debris from the field are potentially effective measures to prevent the formation in plant tissue or the accumulation of microsclerotia in the soil. The effect of those measures depends on the numbers of microsclerotia formed on both aerial and subterranean plant parts. When most of the production of microsclerotia takes place on the aerial plant parts, then a haulm treatment could affect the increase of the inoculum potential of *V. dahliae*. For an accurate estimation of the effect of treatments of potato haulm on the formation of microsclerotia, the density of microsclerotia on the various plant organs, the dry weight ratio of the plant organs, and the total number of microsclerotia per plant organ and per plant should be quantified. These data and the effects of haulm treatments should also be known for different crop species and cultivars to study population dynamics.

In practice, the potato haulm is killed at various stages of maturity, depending on the purpose for which the crop is grown. Davis et al. (1983) found a sharp increase of the density of the microsclerotia when the harvest was delayed. The harvest time may interact with haulm treatments and this interaction should be investigated.

Because of the very high numbers formed in plant tissue, microsclerotial formation is not easily quantified. Image analysis may offer a possibility to improve the capacity to count microsclerotia.

In a crop situation, the balance between formation and mortality of microsclerotia determines the soil inoculum density. Moreover, the effect of the inoculum density on a crop can vary, depending on the provenance of the pathogen (Tjamos, 1981; Zilberstein et al., 1983b). It is hypothesized that also in Dutch crop rotations, host specificity of *V. dahliae* is important.

The long persistence of microsclerotia and the long time that microsclerotia stay embedded in host debris obscure the effects of treatments in short-term experiments and make a model an attractive and necessary tool to analyse data sets over more years. Modelling may also provide a tool to get an idea of the dynamics in soil inoculum and can predict the consequences of various crop rotations for the development of the soil inoculum density.

In summary, the objectives of this project were:

- to explore the effects of plant roots on the germination of microsclerotia of *V. dahliae*;
- to analyse the formation of microsclerotia and various measures to reduce formation of microsclerotia;
- to develop a model that describes the population dynamics in the soil under various crop rotations.

SECTION 1.4

STRUCTURE OF THE THESIS

In Chapter 2 experiments are described in which the effects were assessed of single plant roots and crops on microsclerotia in the soil. Root observation boxes were used to carry out a quantitative study on the germination of microsclerotia. Root densities were measured in a field experiment to calculate the fraction of the soil volume affected by plant roots in a crop situation. In Section 2.2 the influence of various crops on the population of microsclerotia in the soil is calculated from results from the experiments reported in Section 2.1 and from two additional experiments.

In Chapter 3, a method is described to count microsclerotia in plant material by image analysis.

Chapter 4 deals with the formation of microsclerotia on various crop species and cultivars. In the experiments described in Section 4.1, plants of various crop species and potato cultivars were inoculated or grown in infested soil to quantify the formation of microsclerotia. In Section 4.2, the results of two greenhouse experiments are described in which the production of microsclerotia was measured in different parts of two potato cultivars at two harvest dates.

Chapter 5 reports on an experiment in which changes in soil inoculum density were measured as influenced by growing ten different crop species and cultivars during two subsequent years; crop yields are reported as well. Soil was initially infested in two densities with two isolates of *V. dahliae* obtained from soils with different cropping histories. In the third year a susceptible potato cultivar was grown to analyse the effects of the crop species and cultivars, grown in the previous two years, and of the initial infestation level.

In a field experiment which is reported in Chapter 6, the effect of removal of crop debris on soil inoculum level of *V. dahliae* was investigated for two isolates. Changes in inoculum density were recorded for a period of three years for different crop sequences including a susceptible potato cultivar, field bean and barley.

In Chapter 7, the results of four pot experiments are described in which the formation of microsclerotia on potato haulm of various lengths, covered with soil or kept on the soil surface, was studied. Formation of microsclerotia with these treatments was compared with formation of microsclerotia after haulm killing with a herbicide or by heating, on two harvest dates.

In Chapter 8, the dynamics of the soil inoculum density are approached theoretically with a quantitative model. An equation was developed that models the inoculum densities of *V. dahliae*

over long time spans (years) based on inputs of initial inoculum densities and cropping sequences. The number of systemic infections of plant roots during crop growth was related to soil inoculum density. In turn, formation of microsclerotia in debris and reduction of the amount of the debris of different crops were related to the number of systemic infections. Finally, a gradual release and mortality of microsclerotia in the soil were included to calculate subsequent inoculum densities in the soil. The formation of microsclerotia and the production of crop debris were related to a series of observations on soil inoculum densities, to correlate soil inoculum density to crop yield and subsequent inoculum densities. The resulting equation is based on biologically and ecologically meaningful principles and measurable parameters.

The thesis ends with a general discussion focusing on the potential of agronomic measures studied to control the pathogen.

CHAPTER 2

EFFECT OF PLANT ROOTS ON THE GERMINATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE*

SECTION 2.1

EFFECT OF PLANT ROOTS ON THE GERMINATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE*

I USE OF ROOT OBSERVATION BOXES TO ASSESS DIFFERENCES AMONG CROPS

L. Mol and H.W. van Riessen

Summary

Root observation boxes were used to study the effects of hosts and non-hosts on the germination of microsclerotia of *V. dahliae*. The effects of roots on microsclerotia were examined within a radius of 1 mm around the root tip. Host plants such as potato and field bean induced a higher percentage of germination of the microsclerotia than a non-host such as barley. A susceptible potato cultivar stimulated germination more than a resistant cultivar. The germination percentage and the number of hyphae per microsclerotium decreased with distance from the root surface regardless of the plant species or cultivar.

Introduction

Microsclerotia (MS) of *Verticillium dahliae* (Kleb.) remain dormant in the soil but are stimulated to germinate by root exudates. The infectious hyphae that emerge from MS penetrate roots mainly in the areas of cell differentiation and in the root hair zone (Schnathorst, 1981). Only a very small proportion of the hyphae will successfully infect the root. If a species or cultivar can be systemically infected by *V. dahliae* it is called a host. However, large numbers of colonies per unit root length have been found in non-host plants as well in host plants (Evans & Gleeson, 1973). *Verticillium dahliae* primarily colonises the root cortex near the root tip; the maximum density of penetration is 1 cm from the root apex (Gerik & Huisman, 1988).

Microsclerotia of *V. dahliae* can germinate more than once, but eventually become exhausted (Farley et al., 1971). Since both hosts and non-hosts can induce germination of MS (Schreiber & Green, 1963), a possible strategy to control *V. dahliae* might be to grow non-hosts. Indeed, inducing MS to germinate without producing abundant propagules after plants have been

infected, may be a major factor in the Verticillium wilt control, that has often been reported when non-hosts are used in rotations (Schnathorst, 1981).

The reasons for the root's greater stimulating effect on microsclerotia around its tip compared with other sites along the root axis may be that exudates are excreted from the zone of root elongation (Rovira & Davey, 1974; Curl & Truelove, 1986) and that there are relatively low densities of organisms competing for nutrients here (Olsson et al., 1987). Fitzell et al. (1980) found that the density of root colonisation by *V. dahliae* increased up to 20 mm from a growing root tip in wheat (*Triticum aestivum*) and thornapple (*Datura stramonium*), but Gerik and Huisman (1988) showed that in cotton the occurrence of colonies of *V. dahliae* did not increase beyond 5 mm from the root tip. Both these studies (Fitzell et al., 1980; Gerik & Huisman, 1988) give only an indication of the number of colonies at the root surface. Because of possible interactions between MS, root exudates, and rhizosphere microorganisms, the colonisation of the root does not necessarily give a good indication of the effect of a root on the germination of MS in the soil. After germination most of the hyphae of the MS stop growing and die.

To understand the contribution of the induction of MS germination by plant roots to the control of the pathogen, quantitative information on the influence of crop roots on the germination of MS in the soil is needed. This paper reports on research to address this by measuring the effect of single root tips on MS in soil non-destructively. The results of two experiments in which the germination of MS was measured as influenced by host plants and non-host plants are presented.

Materials and methods

Production of microsclerotia

Green potato stems from the field were cut so they were long enough to be contained upright in an Erlenmeyer flask. The stems were autoclaved for 20 min. at 120°C. Two-week old sporulating *V. dahliae* culture on PDA slants, isolated from potato, was blended with sterile water.

The stems were dipped in the solution, and were incubated under sterile conditions for four weeks at 22°C. By the end of this period the stems were completely covered with microsclerotia. The stems were air-dried, ground and kept in a dry place at room temperature until used.

Root observation boxes

To study the effect of roots on the germination of MS, wooden root observation boxes (24x18.5x5 cm) with one removable transparent acrylate side were constructed (Fig. 1). Their bases were perforated to allow free passage of water.

Ground potato stem material containing MS of *V. dahliae* was wet-sieved with tap-water through a 125 μm and a 38 μm sieve. The residue on the 38 μm sieve was collected in a minimum amount of water, and the concentration of the MS suspension was calculated by counting the MS under a microscope. The MS were added to water-agar (7.5 g l^{-1}) maintained at a temperature between 35 and 40°C, and the mixture was stirred thoroughly. Using a syringe, an agar layer 2 mm thick containing the MS (on average 15 MS mm^{-2}) was built up on the transparent plate.

Before the transparent plates were screwed back onto the boxes, unsterilised moistened sandy soil (pH: 5.8, 9.1% organic matter) was put into the boxes to within 3 mm of the top. This uppermost 3 mm was then filled in with ground dry soil, to ensure good contact (without air spaces) with the agar layer containing the MS. An unsterilised soil was used, to maintain the effect of microbial activity on MS (Emmaty & Green, 1969). The boxes were placed on a slant, to ensure contact between plant roots and the agar layer. Light penetration through the transparent wall was prevented.

The prepared boxes were pre-incubated for 7 days under the conditions described under 'Experiments', to allow an equilibrium to be reached between the micro-life in the box and the agar layer. During the incubation and the experiments, the boxes were moistened by placing them on a bed of wet sand and by watering from above.

Optimisation of the method

The method described above was developed and refined in accordance with various preliminary experiments. In one such experiments, the plant material containing the MS was not sieved and there appeared to be many aggregates of MS and extensive growth of micro-organisms from plant tissue particles. This made it difficult to observe the germination of MS. Tests showed that the problem of adherent micro-organisms could be decreased by wet sieving the plant material. This technique also removed small MS and large aggregates of MS.

A soft water-agar (7.5 g l^{-1}) was used to fix the MS to the inner side of the transparent plate. We experimented in Petri dishes to find out if the germination of MS was affected by the initial temperature of the agar. We tested agar at two temperatures: 33°C or 40°C and found no difference in the germination of the MS.

In another preliminary experiment, four methods of adding the MS were tested in Petri dishes: A) pouring the liquid agar over the MS on the base of the dish; B) putting the MS on the top of the solidified agar; C) putting the MS on the top of the liquid medium, and D) pouring the agar after suspending the MS. Treatment D showed the highest germination percentage of the MS, and had the best distribution of the MS over the surface of the dish. Propagules near the surface had a higher germination percentage than MS deeper in the agar.

After the observations on germination, Petri dishes were filled with a sandy soil and the treatments were tested to ascertain the visibility of fungal hyphae and germination of MS. It appeared that hyphae and germinated MS were very visible in treatments B and D. Some space between the MS and the soil background was necessary to enable the MS to be distinguished from soil particles. Therefore, it was decided to use treatment D as the standard method for the preparation of the agar layer inside the boxes.

Experiments

Twenty boxes were placed in a growth chamber at 22/15°C day/night with a 14 h thermo- and photophase. After the pre-incubation, two 10-day-old seedlings or two pre-rooted potato sprouts were planted per box. Four crops were grown in four replications: potato (*Solanum tuberosum*) cv. Element (a sensitive cultivar), potato cv. Mirka (a tolerant cultivar) (Scholte & s'Jacob, 1990), barley (*Hordeum vulgare*) cv. Prisma (a non-host), and field bean (*Vicia faba*) cv. Victor (a host). Four boxes without a crop were used as a control. Two weeks after planting, the root system had reached the bottom of the box and measurements were started. The experiment was repeated in five replications with a different observer (Experiments 1 and 2, respectively).

Non-destructive observation of roots and microsclerotia

Five root tips were examined per box. To avoid the influence of excessive moisture at the bottom of the box, and the influence of fluctuations of moisture near the top of the box, roots in the centre of the box were selected for examination. Because it was known that the largest effects would be measured close to the root surface, a zone of only 1 mm in radius around a root tip was examined. In the control, measurements were taken at five spots with a radius of 1 mm, randomly in the middle of the box.

A binocular-microscope (magnification 100x) was used to count the number of hyphae per MS and to measure the distance between a germinated or a non-germinated MS and the root surface (Fig. 2). Per root tip 45 MS were examined. Distances were measured with a measuring eyepiece in units of 10 μm. A long distance object-lens was required to be able to focus at the roots and MS under the 3 mm thick acrylate plate.

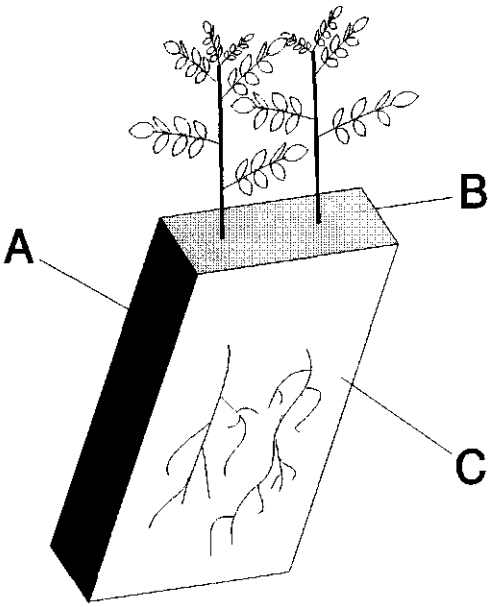

Fig. 1. A root observation box (24x18.5x5 cm) constructed of wooden sides (A), with one transparent acrylate plate (C). The box is is filled with unsterilised soil (B).

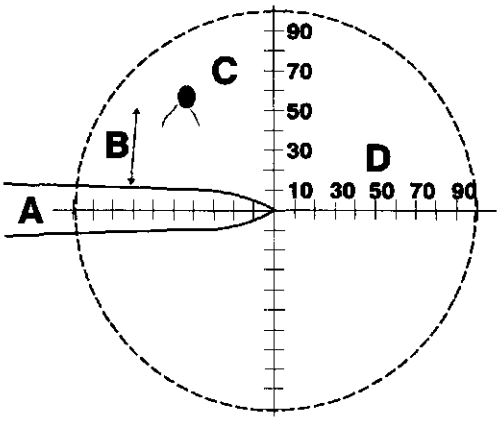

Fig. 2. Schematic representation of the view through the microscope. The centre of the measuring eyepiece is set at the root tip (A). In a circle of radius 1 mm, the distance of the MS (C) to the root surface (B) was measured in units of 10 μm (D). The number of hyphae per microsclerotium was counted.

Results

The two experiments produced very comparable results (Table 1). The highest levels of germination were found for potato cv. Element and field bean, followed by potato cv. Mirka and barley, and with the control lagging far behind. The most hyphae per germinated MS were found in potato cv. Element and field bean. The control had the fewest hyphae per MS, but for this parameter differences between treatments were not large. The number of hyphae per MS, indicating the overall effect of the root, was more than three times higher in potato cv. Element

than in the control. Field bean, potato cv. Mirka, and barley also stimulated the number of hyphae per MS, but the effect was least in barley.

Linear regression on the distance of the root surface showed that both the percentage of germinated MS and the number of hyphae per MS decreased significantly ($P < 0.01$) with distance from the root tip (Figs. 3a and 3b). The intercepts of the lines for the crops were significantly different ($P < 0.025$), but there were no statistically significant differences among the regression coefficients of the four crops.

Table 1. Effects of roots on the germination of microsclerotia (MS) within a radius of 1 mm around the root tip in Experiments 1 and 2.

	Potato		Field bean	Barley	Control	LSD (P=0.05)
	'Element'	'Mirka'				
MS germinated tip^{-1} (%)						
Exp. 1	37.9	26.2	38.7	29.7	15.3	10.7
Exp. 2	48.7	37.6	43.6	35.4	20.5	10.4
Mean	43.9	32.7	41.4	32.9	18.2	6.8*
Number of hyphae (germinated MS)$^{-1}$						
Exp. 1	1.61	1.52	1.43	1.39	1.40	0.28
Exp. 2	1.44	1.53	1.31	1.13	1.15	0.15
Mean	1.52	1.53	1.36	1.25	1.27	0.14*
Number of hyphae MS^{-1}						
Exp. 1	0.66	0.44	0.56	0.43	0.23	0.26
Exp. 2	0.71	0.58	0.57	0.40	0.24	0.16
Mean	0.69	0.52	0.56	0.41	0.23	0.13*

*LSD values for the means are calculated from an analysis of variance based on the combined data of Expts. 1 and 2

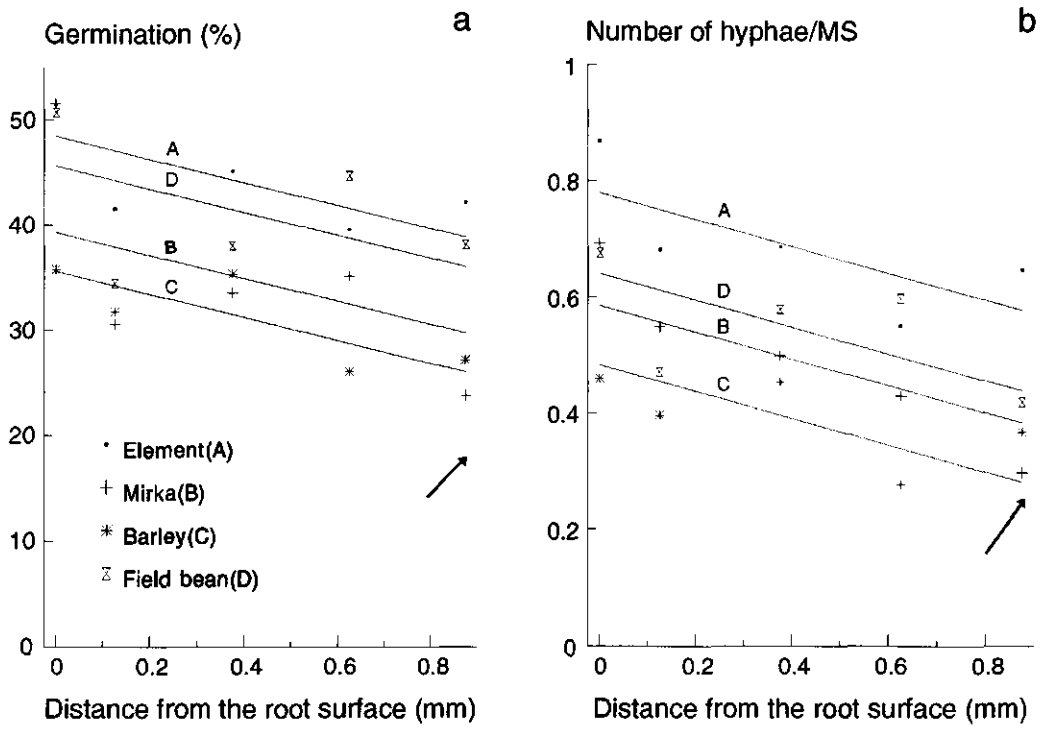

Fig. 3. Relation between the distance from the root and the percentage germinated micro-sclerotia (a) or the number of hyphae per microsclerotia (b) for the four crops tested. Before the regression was calculated, MS were grouped into five distance classes from the root: 0, 0.01-0.25, 0.26-0.50, 0.51-0.75, and 0.76-1.00 mm. The medians of the classes were taken as the independent variable. There was a statistically significant decrease over the distance ($P<0.01$). Intercepts also differed statistically significantly among crops ($P<0.025$), but slopes did not. The arrows indicate the level of the control.

Discussion

From the above it is clear that this experimental method using root observation boxes gives a quantitatively reproducible description of the influence of plant roots on MS of *V. dahliae*. The method has two shortcomings: the MS do not make actual contact with the soil, and roots next to the transparent acrylate side are not fully covered with soil. Because of this, one cannot be sure that the effect as measured is similar in nature and of comparable level to that occurring in

real life in the soil (Lockwood, 1964). However, the method has the advantage that the influence of the roots on the MS can be easily and directly measured in space and time.

In the control treatment, with no plant, some germination was observed, though this was very much lower than that in all treatments with plants, however, germination was much higher. In accordance with other authors (Schreiber & Green, 1963; Emmatty & Green, 1969; Fitzell et al., 1980; Schnathorst, 1981; Zilberstein et al., 1983b), we found that the stimulation of germination of MS of *V. dahliae* by plant roots is to some extent unspecific for crops. This may be related to the demonstrated effect of various carbohydrates (Emmaty & Green, 1969; Zilberstein et al., 1983b) and amino acids (Emmaty & Green, 1969) on the induction of germination.

The germination in the control treatment in our experiments was high, but is comparable with the results obtained by Schreiber and Green (1963) who used an agar disc technique. No data are available about the germination of MS in the soil under field conditions without the influence of a plant. The germination we found in the control could have been caused by components from the agar layer, by a lack of antagonistic organisms in the agar, or by soluble compounds of the soil solution, but not by fresh specific exudates from plant roots.

Roots of host crops (potato, field bean) showed a stronger stimulating effect on germination of MS per root tip than the roots of the known non-host crop (barley). This confirms the results of Schreiber and Green (1963) and Fitzell et al. (1980). There were clear differences between the potato cvs Element and Mirka. Part of the sensitivity may be expressed at the level of the stimulation of the germination of the MS. The composition of the exudates might be a determining factor. Genetic research has shown that the host genotype may play a major role in determining the characteristics of the rhizosphere populations of bacteria in wheat, largely through control of the quality of the root exudates (Curl & Truelove, 1986). Differences between crops are even clearer when the effect of crop roots (or of their exudates) over distance are considered (Figs 3a and 3b). Although the level of germination at the root surface of barley was rather high, at a distance of 1 mm it had fallen to that of the control treatment. In the Verticillium-susceptible crops potato cv. Element and field bean the germination at a distance of 1 mm from the root surface was still more than double that in barley and in the control. So, the influence of these crops will be perceptible at a distance of more than 1 mm. From this we infer that the results of germination and the number of hyphae per MS of potato cvs Element and Mirka, and field bean underestimate the influence per root tip compared with barley or the control. An effect over a certain distance will be important for the reduction of the MS population in the soil caused by the plant roots. Hyphae beyond a critical distance are likely to lyse before they reach the root suface (Fitzell et al., 1980), because *Verticillium* spp. have

shown to be very poor saprophytes in the soil (Schnathorst, 1981). If the concentration of the exudates is the determinant in stimulating MS to germinate, the relation should be non-linear. It is remarkable that we did not get a better fit with a function relating the effect on the MS to the inverse distance from the root surface. Perhaps, a high microbial activity reduced the effect of the exudates close to the root, leading to a flattening of the line. Additionally, the area observed around the root tip may have been too small to yield sufficient data to establish a non-linear relationship. The correlation for each single crop was not very high and a change of one data point may have a considerable effect on the shape of the line.

Microsclerotia require no exogenous source of nutrients for germination *in vitro* (Green, 1971). Furthermore, it has been shown that germination can be inhibited by high CO_2 concentration, low O_2 concentration (Zilberstein et al., 1983a), and a relatively high soil temperature (Dutta & Isaac, 1979). It is also difficult to draw general conclusions, because just one type of soil is used in the experiments. Research done by Rovira and Davey (1974) suggests that the age and stage of development of the plant, the light intensity, temperature, pH, soil moisture, and O_2 and CO_2 concentrations may indirectly influence the stimulation of the MS qualitatively and quantitatively via their influence on the exudation of plants (Rovira & Davey, 1974). However, from our experiments it is clear that crops differ in their stimulation of the germination of MS of *V. dahliae*; this aspect deserves to be followed up and clarified.

SECTION 2.2

EFFECT OF PLANT ROOTS ON THE GERMINATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE*
II QUANTITATIVE ANALYSIS OF THE LURING EFFECT OF CROPS

L. Mol

Summary

Induction of germination of microsclerotia by exudates from plant roots may be important for the control of *V. dahliae*. Laboratory experiments with root observation boxes were carried out to assess the influence of root tips of seven crop species and cultivars on the germination of microsclerotia of *Verticillium dahliae* in soil under controlled conditions. The root density of crops was measured in a field experiment. The results of the laboratory experiments and the field experiment were combined to estimate the total effect of crops on the population of microsclerotia in the field. Germination of microsclerotia was stimulated by all crops compared to a control without a crop. Among crops, roots of potato cvs Element and Astarte had a larger stimulation effect on microsclerotia than that of potato 'Ostara', pea, flax, sugar beet or onion. The number of hyphae per microsclerotium decreased with distance from the root surface regardless of the crop species or cultivar. Differences in root densities, in the affected root zones and in the stimulation effect on germination of microsclerotia caused large differences among crops in the effect on the population of microsclerotia in the soil. However, growing a crop with the special purpose to reduce the level of *V. dahliae* inoculum in the soil is an inefficient control measure, because only a small part of the total soil volume is affected by roots and the number of hyphae per microscleroium affected is too low.

Introduction

Root exudates of crops can stimulate the germination of microsclerotia (MS) of *Verticillium dahliae* Kleb. in the soil (Schreiber & Green, 1963; Mol & Van Riessen, 1995). However, only a very small portion of the hyphae will successfully infect the root. Microsclerotia of *V. dahliae*

can germinate more than once, but eventually become exhausted (Farley et al., 1971; Ben-Yephet & Pinkas, 1977). Crops could affect the soil population of MS through stimulation of MS germination by its roots. Schreiber and Green (1963) found differences between the effects root exudates of tomato and wheat on MS germination. Using root observation boxes, Mol and Van Riessen (1995) showed similar differences among five crops.

In crops with a high root density and without subsequent proliferation of *V. dahliae* in the root system, the effect of the roots on the MS population may be relevant to reduce the MS density in the soil. Benson and Ashworth (1976) did not find differences in inoculum density between soil adhering to the root system of plants and soil without roots. This suggests that the negative effect of roots on the existing MS population was negligible. Knowledge of effects of roots of crops on the population of MS is important to understand the value of crops to reduce the MS density in the soil.

In two laboratory experiments, the influence of root tips of seven crop species and cultivars has been quantified using the method of Mol and Van Riessen (1995). In a field experiment, the root density of the crops was measured. The results of these experiments and the results of Mol and Van Riessen (1995) are used to calculate the effect of the crops on the MS population.

Materials and methods

Effect of crops on MS

Root observation boxes were constructed and prepared according to Mol and Van Riessen (1995). In these boxes roots grow along a transparent wall, coated with an agar film containing individual MS. Microsclerotia of *V. dahliae* were taken from potato stems (cv. Bintje) from a commercial field of the Department of Agronomy in the autumn of 1990. The MS were scraped off the stems and wet-sieved through a 125 μm mesh and a 38 μm mesh sieve. The residue on the 38 μm sieve was collected. The boxes were filled with unsterilized moistened sandy soil (pH: 5.5, 2.7% organic matter).

Forty boxes were placed in a growth chamber at 22/15°C day/night with a 14 h thermo- and photophase. After a pre-incubation period of 7 days, two 10-day-old seedlings or two pre-rooted potato sprouts were planted per box. Seven crops were grown in five replications: potato (*Solanum tuberosum* cvs Element (a cultivar sensitive to *V. dahliae* (Scholte & s'Jacob, 1990)), Ostara and Astarte), pea (*Pisum sativum* cv. Finale), sugar beet (*Beta vulgaris* cv. Univers), onion (*Allium cepa* cv. Jumbo), and flax (*Linum usitatissimum* cv. Viking). Five boxes without a crop were used as controls. Two weeks after planting, the roots had reached the bottom of the

box and observations on effects of roots on germination of MS were started. Germination was quantified using the method as described by Mol and Van Riessen (1995): counting the number of hyphae originating from a MS and measuring the distance between a germinated or a non-germinated MS and the root surface with a binocular microscope (magnification 100x). The experiment was completely repeated with a different observer (Experiments 1 and 2).

Root density of field crops

Root densities of crops were measured in a field trial on a sandy soil (pH: 5.4, 3.7% organic matter) (Experiment 3). On May 7 ten crops were planted or sown on plots of 4 x 4 m^2: potato cvs Element, Mirka, Ostara and Astarte, spring barley (*Hordeum vulgare* cv. Prisma), field bean (*Vicia faba* cv. Victor), pea cv. Finale, sugar beet cv. Univers, onion cv. Jumbo, and flax cv. Viking. The distance between the rows was 50 cm for potato, field bean and sugar beet, and 25 cm for the other crops. Weeds were removed by hand.

Root samples were taken at the time that a maximum root density was expected. For barley, field bean, pea, and flax this was at the onset of flowering, for onion at the onset of bulbing, for potato when tuber bulking started, and for sugar beet in the first half of August, several weeks after closure of the canopy. Except for potato, samples were taken between plants in the row, and between the rows. Per plot eight cores were taken. For potato the sampling scheme was based on Vos and Groenwold (1986): one data point was the average of two bore holes in the row, two halfway the slope of the potato hill, and two in the furrow between the rows. In Exp. 3 the bore holes halfway the slope of the potato hill were skipped. Two duplicates were taken per potato plot, each of four subsamples.

Samples were taken at three depths: 0-10 cm, 10-20 cm, and 20-30 cm, using an auger with a diameter of 7 cm and a volume of 179 ml. Root samples were washed and the roots were stored in a 15% ethanol solution until analysis. The root length was estimated by the line-intersection method described by Newman (1966), as modified by Tennant (1975), using a 2 cm mesh grid.

Data analysis and calculations

Based on the observations of Fitzell et al. (1980) and Gerik and Huisman (1988) the induction of MS to germinate by the root system of a plant is assumed to diffuse from the root tip. From the results of Expts 1 and 2, the effect per root tip was calculated for each crop tested. The results of Expts 1 and 2 were combined, because both experiments gave similar results. Qualitative linear regression was used to fit the number of hyphae per MS to the distance of the

MS from the root surface. The intercepts and the slopes of the different crop species and cultivars were compared. Before the regression was calculated, MS were grouped into five distance classes: 0, 0.01-0.25, 0.26-0.50, 0.51-0.75, and 0.76-1.00 mm. For the analysis the median was taken to represent each class.

Root lengths of crops of Exp. 3 were calculated per 10 cm soil layer from the means of eight cores per plot. Root lengths for the soil layer 0-30 cm were calculated by adding up the means of the three layers.

For the calculation of the effect of roots on the MS population in the soil, the data of Expts 1, 2, and the results of laboratory experiments of Mol and Van Riessen (1995) were combined with the root density data collected in Exp. 3. The measured values and the mean slopes of Expts 1 and 2 differed from those observed by Mol and Van Riessen (1995) (see Results). To arrive at a valid comparison of crops from current and previous experiments, results had to be adjusted. Results of potato cv. Element and the control treatment were used as references, because they were present in all experiments. In a first set of calculations the mean numbers of hyphae.MS^{-1} as published by Mol and Van Riessen (1995) were reduced to the values of Expts 1 and 2, and the slope of Expts 1 and 2 was used to calculate the variables. In a second set of calculations the mean numbers of hyphae.MS^{-1} from Expts 1 and 2 were increased to the values as published by Mol and Van Riessen (1995), and the slope calculated by them was used for the calculations.

The radius of the rhizosphere (R) of a single root of each crop was defined as the distance from the root at which the influence of the root on MS germination became nil (i.e. was decreased to the level of the control treatment). The linear regression line was used to estimate the radius of the rhizosphere (for formulas and calculations see Appendix A). For each crop species and cultivar the percentage of the soil volume affected by the roots (V) was calculated from the radius of the rhizosphere and the root density determined in Exp. 3 using the formula for a cylinder. Because the effect is measured at the root tip, the thickness of the root is considered to be negligible. The mean effect of plant roots on the MS population in the soil ($\bar{E}$) was calculated by:

$$\bar{E} = c_1 + \frac{2}{3} c_2 . R$$

The formula is the result of the integration of the linear relationship of the effect from the root (c_1 = intercept, c_2 = slope) and the area of a circle over the radius of the rhizosphere (R) (Fig. 1, Appendix A).

Results

The highest levels of MS germination were found for potato cvs Element and Astarte, followed by potato cv. Ostara and the other crop species (Table 1). All crops showed a significantly higher germination than the control. Differences in the number of hyphae per germinated MS were small between crops. The number of hyphae per germinated MS in the control was significantly lower than for most crops. Small differences among crops were found in the number of hyphae per germinated MS. Therefore, the number of hyphae per MS showed similar differences among crops as the MS germination. The susceptible potato cv. Element had the highest values, and all crops showed significantly higher values than the control treatment.

The differences in root density between crops were comparable for the different soil layers (Table 2). The root density to a depth of 30 cm was the highest for barley and flax. In a decreasing order, the sequence for the other plant crops was pea, sugar beet, field bean, potato cvs Mirka, Astarte, Element, and Ostara, and onion, the value of onion being only 25% of the one of barley. Differences among potato cultivars were large. The root density of 'Mirka' was almost double the one of 'Ostara'.

Linear regression showed that the number of hyphae per MS decreased significantly ($P < 0.01$) with increasing distance from the root tip. The intercepts of the lines for the crops were significantly different ($P < 0.05$), but the slopes of the regression lines did not differ significantly. The average slope had a value of -0.49 hypha $MS^{-1}mm^{-1}$.

Table 1. Mean effects of roots on the germination of microsclerotia (MS) within a radius of 1 mm around the root tip in Experiments 1 and 2.

Crop	MS germinated/ tip (%)[a]	Number of hyphae/ (germinated MS)[a]	Number of hyphae/ (MS)[a,b]
Potato cv. Element	33.3 a	1.18 ab	0.40 a
Potato cv. Astarte	29.3 a	1.21 ab	0.36 a
Potato cv. Ostara	18.4 b	1.25 a	0.23 b
Pea	23.1 b	1.25 a	0.29 b
Flax	19.5 b	1.28 a	0.25 b
Sugar beet	20.9 b	1.28 a	0.27 b
Onion	19.2 b	1.26 a	0.25 b
Control	9.3 c	1.12 b	0.10 c

[a]Different letters indicate significant differences between the treatments based on LSD values (P=0.05) of the combined data of Expts 1 and 2.

[b]Number of hyphae / MS = (MS germinated / tip (%) x Number of hyphae / germinated MS) / 100.

Table 2. Root densities ($cm.cm^{-3}$) of 10 crops in various soil layers. Experiment 3.

	Soil layer Depth in the soil (cm)*			
	0-10	10-20	20-30	0-30
Potato cv. Element	1.49 c	1.47 cd	1.67 c	1.54 cd
Potato cv. Astarte	2.31 b	1.93 c	1.65 cd	1.96 c
Potato cv. Ostara	1.36 c	1.13 d	1.09 de	1.19 d
Potato cv. Mirka	2.65 b	1.90 c	2.01 c	2.19 bc
Field bean	2.60 b	2.50 b	1.51 cd	2.20 bc
Barley	4.34 a	3.89 a	4.38 a	4.20 a
Pea	2.53 b	2.75 b	2.61 bc	2.63 b
Flax	4.89 a	3.49 a	2.79 b	3.72 a
Sugar beet	3.04 b	2.38 bc	1.83 c	2.42 bc
Onion	1.00 c	1.24 d	0.80 e	1.01 d

*Different letters indicate significant differences between the treatments based on LSD values (P=0.05).

Expts 1 and 2 differed from the experiments of Mol and Van Riessen (1995) in the effect of roots on the number of hyphae per MS. For the control and potato cv. Element the effect in Expts 1 and 2 was 0.58 and 0.44 times the effect found by Mol and Van Riessen (1995), respectively. For the calculation based on the observed number of hyphae per MS of the current experiments, the mean effects of potato cv. Mirka, field bean, and barley obtained in the latter experiments were multiplied by 0.51 (the average of 0.58 and 0.44) (Table 3A). For the calculation based on the values measured by Mol and Van Riessen (1995) the observed numbers of hyphae.MS^{-1} of the current experiments were divided by 0.51 (Table 3B).

Differences in the mean number of hyphae per MS between crops led to comparable differences in the intercepts, the affected root zones, and the mean numbers of hyphae per MS in the affected soil volume (Table 3). Because of the mathematical integration of the linear decrease of the effect from the root and the affected volume, differences among the crops were smaller when the mean number of hyphae per MS in the affected soil volume was calculated. Differences in both root densities and in the affected root zones caused large differences in the affected soil volume between crop species and cultivars. Differences were largest when the MS were artificially produced. Potato cvs Astarte and Element, pea and flax had the highest proportions of the soil volume affected, followed by field bean, sugar beet, potato cv. Mirka, barley, with onion and potato cv. Ostara lagging far behind.

Table 3. Effect of roots on the soil population of microsclerotia (MS) of *V. dahliae*, obtained after adjusting the level to the values of Expts 1 and 2 (**A**), and adjusted to the level of the values measured by Mol and Van Riessen (1995) (**B**). Transformations are explained in the text.

Crop	Number of hyphae/ MS	Intercept (number of hyphae/ MS)[b]	Affected root zone (mm)[c]	Affected soil volume (%)[d]	Mean number of hyphae in affected volume /MS[e]	Effect on the soil population/ (MS)[f]	Relative differences
A *(adjusted to the level of the values of Expts 1 and 2)*							
Potato 'Element'	0.40	0.63	1.07	5.6	0.28	0.113	110
Potato 'Astarte'	0.36	0.57	0.96	5.7	0.26	0.112	109
Potato 'Ostara'	0.23	0.43	0.67	1.7	0.21	0.105	102
Potato 'Mirka'[a]	0.27	0.50	0.81	4.5	0.24	0.109	106
Field bean[a]	0.29	0.52	0.85	5.0	0.24	0.110	107
Barley[a]	0.21	0.44	0.70	6.4	0.22	0.110	107
Pea	0.29	0.52	0.84	5.8	0.24	0.111	108
Flax	0.25	0.49	0.79	7.3	0.23	0.112	109
Sugar beet	0.27	0.51	0.83	5.2	0.24	0.110	107
Onion	0.25	0.49	0.77	1.9	0.23	0.105	102
Control	0.10	-	-	-	-	0.103	100
B *(adjusted to the level of the values measured by Mol and Van Riessen (1995))*							
Potato 'Element'	0.69	0.78	2.37	27.2	0.41	0.282	121
Potato 'Astarte'	0.70	0.79	2.42	36.2	0.42	0.300	129
Potato 'Ostara'	0.46	0.55	1.36	6.9	0.34	0.239	103
Potato 'Mirka'[a]	0.52	0.59	1.53	16.1	0.35	0.251	108
Field bean[a]	0.56	0.64	1.77	21.6	0.37	0.261	113
Barley[a]	0.41	0.48	1.09	15.6	0.32	0.245	106
Pea	0.57	0.66	1.85	28.5	0.38	0.273	118
Flax	0.49	0.58	1.53	27.3	0.35	0.264	114
Sugar beet	0.52	0.61	1.65	20.8	0.36	0.258	111
Onion	0.48	0.58	1.49	7.1	0.35	0.240	104
Control	0.23	-	-	-	-	0.232	100

[a]Original data are obtained from Mol and Van Riessen (1995). The transformations are explained in the text.

[b]Result of a linear regression. There was a significant decrease over the distance ($P<0.01$). Intercepts of the different crops differed significantly ($P<0.05$), but slopes did not (**A**: slope is -0.49, and **B**: slope is -0.23 hyphae MS^{-1} mm^{-1}).

[c]The affected root zone is the distance from the root to the point at which the regression line obtained with a linear regression reaches the level of the control. See text for further explanation.

[d]Calculated with the formula of a cylinder with the affected root zone (radius) and the root length (Table 2) as measures.

[e]Obtained from integration of the volume around the root within the active distance with the decrease of the number of hyphae from the linear regression.

[f]The mean number of hyphae in the affected soil volume combined with the effect of the control in the volume not affected by the root.

Based on the observations of the current experiments, the effect of plant roots on the soil population of MS was highest for potato cv. Element followed by cv. Astarte, flax, pea, field bean, barley, sugar beet, potato cvs Mirka and Ostara, and onion. Relative to the control, potato cv. Element had an additional effect of 10% whereas cv. Ostara and onion had an additional effect of only 2%. When the values were adjusted to the values observed by Mol and Van Riessen (1995) the order of the relative differences was comparable with the former calculation, but absolute differences among crops were larger.

Discussion

In the current experiments the effects on germination of MS were lower and the absolute differences between crops were smaller than the differences and levels measured by Mol and Van Riessen (1995). However, the trends in both sets of experiments were the same and the relative differences between effects of crops were also the same in all experiments. The lower germination levels of MS in the current experiments may be ascribed to the lower vitality or to latency of the MS. The MS used in Expts 1 and 2 were collected from field stems and were older than the MS used by Mol and Van Riessen. Older MS have a lower germination level (Ben-Yephet & Pinkas, 1977). The soil used was different between the sets of experiments and may also have affected the level of germination, e.g. by different levels of soil fungistasis.

It is unclear whether the conditions in the root observation boxes are representative for field conditions. The calculated values are to a large extent influenced by the value of the affected root zone. The levels of the slopes of the lines and the mean numbers of hyphae per MS from Expts 1 and 2 are considered the ones most representative for a field situation, because the MS used in these experiments were produced in the field. The MS used by Mol and Van Riessen were produced artificially in the laboratory. Although much larger than calculated from Expts 1 and 2, the effect calculated with the artificially produced MS is still too small to contribute significantly to the control of the MS population in the soil. The main reasons for the small effects are the low number of hyphae per MS when a MS is stimulated to germinate by a root and the small percentage of the soil volume affected by the roots. Even when the root density is comparatively high, the soil volume influenced is a limiting factor. Because of this, the elimination of MS by germination will be insignificant and growing a crop with the special objective to control *V. dahliae* will be of little value. Nevertheless, the relative differences between crops show that one crop (potato cv. Astarte) can have a nine-fold effect on germination compared to another crop (onion or potato cv. Ostara). Probably, differences

among crops may change with the composition of the MS population in the soil. An influence of the MS source on germination of the MS has been observed for various crops (Zilberstein et al., 1983b; Krikun & Bernier, 1987).

The results of Expts 1 and 2 show the situation at a well defined age of the plant. During plant growth and development the germination of MS could change as a result of changes in plant properties and root environment. However, Huisman (1988a, 1988b) showed that the colonisation of roots with *V. dahliae* was fairly constant over the season under different environmental conditions.

Gilligan (1979) and Ferriss (1981) calculated the radius at which roots influence organisms in the soil based on the probability of infection of the root and the inoculum density in the soil. Their proposed equations can be used for modelling colony incidence on plant roots but are not sufficient to calculate the effect of plant roots on the population of MS in the soil. For that purpose, properties of the MS population in the soil as calculated in this paper and the source of the MS (Zilberstein et al., 1983b) have to be included. Moreover, knowledge of the distance at which a hypha of *V. dahliae* can reach and infect the cortex of a root is necessary to calculate the inoculum reducing effect of plant roots.

Expts 1, 2, and 3 show that potato cultivars differ in their effect on the germination of MS and also in root length. The intercepts as shown in Table 3 indicate the level of germination at the root surface. At that place the hyphae will have the highest chance to colonise the root. So, the intercept could give an indication of the maximum chance of a plant root to be colonised by *V. dahliae*. In combination with the root length the maximum probability of infection could be calculated. Because a longer root passes along a higher number of MS the differences in root density could explain differences among potato cultivars in the chance to become infected. For other crops only one cultivar was included in the experiments. One should be aware of possible differences among cultivars of the crops in the germination of MS and in root length.

Effects as shown in Table 3 depend to a large extent on the level of the spontaneous germination in the control treatment and the vitality and latency of the MS population in the soil. If the spontaneous germination without the influence of a plant is lower the effect of roots on the MS population will become more important. Therefore, verification of the results in the field is needed, but is very difficult because no techniques for observation are available yet.

Appendix A

Formulas and parameters

$$E = c_1 + c_2 . r$$

$$R = \frac{c_0 - c_1}{c_2}$$

$$V = \pi . R^2 . L . 100$$

$$S = \pi . r^2$$

$$\bar{E} = \frac{1}{S} \int E . dS$$

$$= \frac{1}{S} \int_0^R . \int_0^{2\pi} E . r . dr . d\varphi$$

$$= \frac{2\pi}{\pi . R^2} . \int_0^R E . r . dr$$

$$= \frac{2}{r^2} . \int_0^R (c_1 + c_2 . r) . r . dr$$

$$= \frac{2}{r^2} . \int_0^R (r . c_1 + r^2 . c_2) . dr$$

$$= \frac{2}{r^2} . [\frac{1}{2} . c_1 . r^2 + \frac{1}{3} . c_2 . r^3]_0^R$$

$$= c_1 + \frac{2}{3} . c_2 . R$$

E = number of hyphae.MS^{-1};

$\bar{E}$ = mean number of hyphae.MS^{-1} in the affected root zone;

c_0 = measured number of hyphae.MS^{-1} in the control;

c_1 = intercept obtained from linear regression (hyphae.MS^{-1});

c_2 = slope of the regression line (hyphae.MS^{-1}.mm^{-1});

r = distance from the root surface (mm);

R = radius of the rhizosphere (mm);

V = affected soil volume (%);

L = root density ($mm.mm^{-3}$);

S = area of a circle (mm^2).

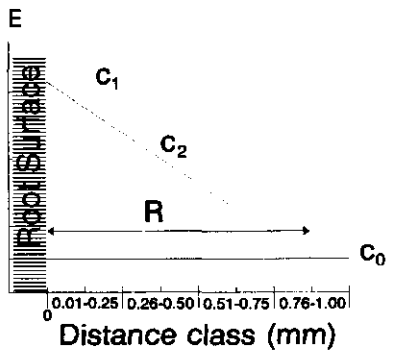

Fig. 1. Schematic representation of the meaning of the parameters used in and obtained from the linear regression.

CHAPTER 3

QUANTIFICATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE* IN PLANT MATERIAL BY IMAGE ANALYSIS

CHAPTER 3

QUANTIFICATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE* IN PLANT MATERIAL BY IMAGE ANALYSIS

L. Mol and E.M.J. Meijer

Summary

A procedure for the quantification of microsclerotia of *Verticillium dahliae* with an image analysis system was compared with counting by eye. Colonised potato plant material was used from plants grown in pathogen-free soil in a greenhouse and from twelve crops (including four potato cultivars) grown outdoors in pots filled with pathogen-free soil under natural conditions.

The values obtained from the potato material from the greenhouse were comparable for both methods. Variation in the results mainly resulted from sampling errors. The numbers of microsclerotia in plants grown outdoors were overestimated by image analysis for most crops. The source of the error was related to the presence of plant and soil particles that did not discolour during boiling of the samples in sodium hydroxide. Image analysis was a suitable and reliable method for assessing the number of microsclerotia only in potato haulm samples from plants grown in pathogen-free soil in the greenhouse.

Introduction

Verticillium dahliae Kleb. forms microsclerotia on senescing plant material. Direct estimations of the reproduction of *V. dahliae* on plant parts by estimating the area colonised with MS or by counting the MS seem unreliable for quantitative analysis. Problems are the very high numbers of MS that can be produced per unit plant material and the large differences within and among plant parts (Menzies, 1970).

Ben-Yephet & Szmulewich (1985) assessed the numbers of MS in potato debris by direct counting in small subsamples. Slattery (1981) and Davis et al. (1983) plated ground potato stem material on a semi-selective medium. Their method proved to be reliable, but they could not distinguish between colonies from single or aggregated MS in the debris, because grinding does

not sufficiently disaggregate microsclerotia. Moreover, plating of plant debris is time and space consuming.

In this paper counting by image analysis (IA) is compared with counting by eye for its suitability to quantify the MS production in plant material grown in a greenhouse (Source 1) and outdoors (Source 2).

Materials and methods

Plant material

For Source 1, potato (*Solanum tuberosum*) plants (cvs Element and Mirka) were infested by immersing rooted sprouts in a blended culture suspension of *V. dahliae* before planting in pure quartz sand or potting compost. Plants were grown in the greenhouse and no other pathogens were found in the plants. Colonised plant material was collected from plants harvested 72 days after planting (green and immature) or 113 days after planting (stems were senescing and close to maturity).

For Source 2, 12 crops were grown in six replications: potato (*Solanum tuberosum* cvs Element, Mirka, Ostara and Astarte), pea (*Pisum sativum* cv. Finale), sugar beet (*Beta vulgaris* cv. Univers), onion (*Allium cepa* cv. Jumbo), flax (*Linum usitatissimum* cv. Viking), spring barley (*Hordeum vulgare* cv. Prisma), field bean (*Vicia faba* cv. Victor), spring wheat (*Triticum aestivum* cv. Minaret), and spring rape (*Brassica napus* cv. Petranova). The crops were grown in 20-l pots filled with a mixture (1:1 by volume) of potting compost and clay soil in which never a crop was grown. Pots were placed in the open air under natural day length and temperature, and were wrapped in insulating foil to prevent heating of the pots by solar radiation. Plants were infested by mixing MS of *V. dahliae* with the soil. Aerial plant parts, stubble and roots were harvested and collected as described by Mol (1995). After the harvest the plant material was air-dried.

Preparation of the samples

For both sources, the air-dried plant material was dry ground with a mill accommodated with a 1 mm mesh sieve. Samples were bleached by boiling for 20 min in 25 ml 1.0 M NaOH. During bleaching almost all plant material discoloured, but MS remained black as well as some particles of the plant material. Two methods of counting were used: counting by eye and counting by IA.

Counting microsclerotia by eye

For counting the MS by eye in the potato plant material from Source 1, the solution of a 20-25 mg bleached sample was filtrated over a Büchner-funnel with a radius of 50 mm. The filter paper with the sample was subsequently dried at room temperature and stored until counting. The MS in the material from the 12 crops from Source 2 were counted in the same bleached sample as used for counting by IA. Before counting, the filter paper with the sample was put on a 0.5 cm mesh grid, and rewetted. A stereo dissecting microscope (magnification 24x) and a hand counter were used to count the MS in the whole sample. A part of the sample was counted when the number of MS counted exceeded 1000.

Counting microsclerotia by image analysis

A SUN-based IA system; GOP 302 (Context Vision Sweden) running under UNIX was used to count the MS automatically. MicroGOP (Context Vision Sweden) was used as a software package, containing most routines necessary for image processing, scan stage control and other level algorithm calls (C, UNIX-shells). After bleaching a 10-15 mg sample, the solution was filtered over a modified Büchner-funnel with an area of 22x22 mm^2. The filter paper with the sample was dried at room temperature and then stored until counting. Samples were not rewetted before scanning because the contrast was better with dry samples. On a white plate of 24.5x24.5 cm^2, 49 samples were glued with water soluble glue in a 7x7 square pattern. Between two samples a free space was kept of 7 mm. This free space was necessary to scan the sample borders. The plate with the samples was put on an 8 inch scan stage table (Marzhauser), computer controlled by a programmable scan stage controller SSCO2 (IDUNA). The scan stage was part of a Stabiplan microscope (Leica) fitted on a marble table and equiped with a M420 Macroscope (Wild). With the 1:5 macrozoom objective at position 18, images of 6 mm in diameter were obtained. A 2/3 inch CCD black & white camera (Fujitsu) was used with a resolution of 582 x 500 pixels plus a 0.4 inch photo-tube to attach it to the M420. An additional cold-light source KL1500 (Scott) with a glass fibre ring illuminator was used to obtain higher contrast and larger depth of field. The microscope magnification was a compromise between the required scan area and resolution; four images in both x- and y-directions were necessary to cover one sample completely. The quadrants were automatically positioned under the microscope by the scan stage which sequentially meandered through all the samples on the object carrier (white plate). Immediately after scanning the image was analysed by the programme to detect only those black particles of the size of MS. This was done by means of a RANK (MAXMIN) filter which made little particles disappear in the image. The obtained image was subtracted from the original image so that the resulting image showed the sample

without shading and with clear tiny spots. One series of 49 samples (i.e. 16 x 49 = 784 images) took nearly 5 hours to scan and analyse. Because it was not necessary to be stand-by, the analysis ran mostly at night or at off-time. After analysis one could print out the number of MS per image, per sample or per plate.

Numbers of microsclerotia in samples from Source 1 obtained by the two counting methods, were compared by linear regression analyses. The results of the samples from Source 2 were analysed by analysis of variance. Pairwise comparisons between the counting methods were made by LSD.

Results and discussion

The IA method gave reliable countings since counting twice a set of the same bleached subsamples of Source 1 resulted in equal values (R^2= 0.99; Fig. 1). In samples from Source 1 obtained from immature or mature plants, IA counting agreed with counting by eye, when separate subsamples were taken for the two methods (R^2=0.93; cv=16%; Fig. 2). A number of samples from Source 1 in a range of 0-1,000 MS per sample were subsampled twice and counted by IA (Fig. 3). There was a high correlation between the first and second counting (R^2=0.85; cv=17%). With an increasing MS density in the sample the standard deviation became higher, but the coefficient of variation remained the same over the whole range. The mean of all numbers counted in the first series of subsamples was very close to the mean of the numbers counted in the second subsample. After 13 times repeated sub-sampling of one source of plant material with a low ($7x10^3$ $MS.g^{-1}$) and one with an intermediate density ($32x10^3$ $MS.g^{-1}$), the coefficients of variation were 13% and 18%, respectively. So, a major part of the variation in the results of counting can be ascribed to sampling errors. When this sampling error is considered a lower coefficient of variation cannot be expected when counting by eye and by IA are compared. It can be concluded that determination of MS in plant material of potato was very accurate for plant material from Source 1.

In samples from Source 2, only for the aerial parts of potato cvs. Element and Ostara, and barley a good correlation (albeit a small overestimation) existed between counting by eye and counting by IA, but for the other crops, counting by IA showed a significant over-estimation of the number of MS compared to the values obtained by eye (Table 1). For Source 1, plants were grown in quartz sand. This soil type is easy to separate from subterranean plant parts and did not turn dark after boiling in NaOH. For Source 2, plants were grown in a mixture of clay soil and potting compost. This soil was very difficult to remove from the subterranean parts. Soil

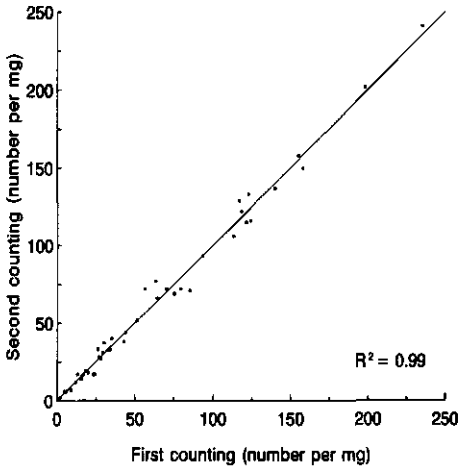

Fig. 1. Relation between the values obtained after counting the same series of prepared samples twice by image analysis. The line represents a 1:1 relationship. Source 1.

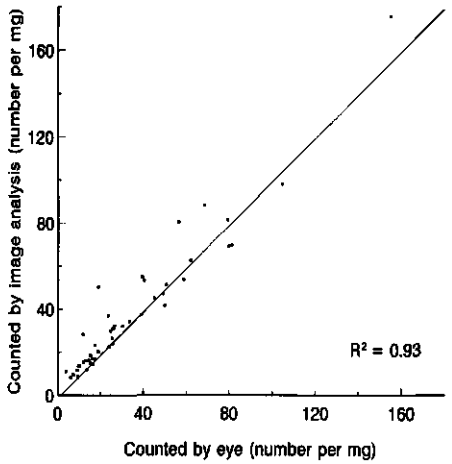

Fig. 2. Number of microsclerotia counted by eye related to the number counted by image analysis. The line represents a 1:1 relationship. Source 1.

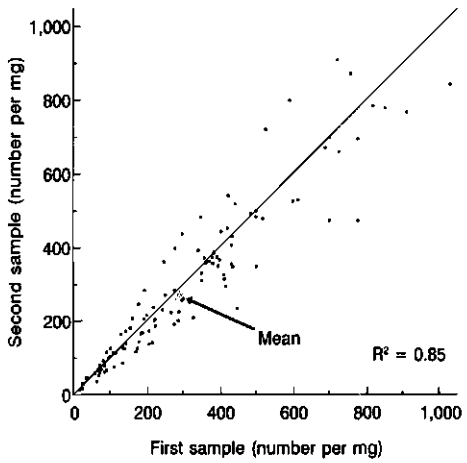

Fig. 3. Relation between the numbers of microsclerotia found in a first subsampling of a set of 123 potato plant material samples and the numbers of MS found in a second subsampling of the same set. The line represents a 1:1 relationship. Source 1.

Table 1. Number of microsclerotia per mg aerial plant material, stubble and root of 12 crops, counted by eye and by image analysis (IA). Source 2.

Crop	Microsclerotia per mg								
	Aerial parts			Stubble			Root		
	eye	IA		eye	IA		eye	IA	
Potato 'Element'	131	158	ns	37	96	***	9	49	**
Potato 'Ostara'	94	115	ns	34	61	***	6	40	**
Potato 'Astarte'	47	86	***	15	62	***	3	23	***
Potato 'Mirka'	34	76	***	12	62	***	6	66	**
Flax	26	34	***	-[a]	-[a]		6	33	**
Field bean	4	43	***	3	17	***	2	36	***
Pea	10	46	**	5	87	**	3	25	***
Barley	14	20	ns	19	61	***	5	59	***
Onion	3	44	***	-[a]	-[a]		4	24	**
Wheat	3	11	***	5	53	***	4	64	**
Sugar beet	4 [b]	66	b,**	4 [c]	146	c,***	1	25	**
Rape	2	17	***	4	23	***	3	31	***

*, **, ***, Results obtained with the image analysis system significantly differ from countings by eye at $P<0.05$, $P<0.01$, and $P<0.001$ respectively; ns= not significant.

[a]No stubble left with the harvest methods used.

[b]Results are from dead leaves.

[c]Results are from leaves and epicotyl.

particles did not always discolour after boiling with NaOH, whereas also the plant material itself did not always lose its colour. Presumably, these are the main reasons for the overestimation. The stubble (green leaves and epicotyl) of sugar beet turned dark after boiling. It is obvious that the high numbers in those samples are unrealistic.

In aerial material of the plant species tested that did not show difference between the methods also some non-MS particles were counted, but the number of MS was so high that this did not cause an unwarranted deviation. So, the use of the IA system is only possible if the relative number of dark particles in the sample after boiling other than MS of *V. dahliae* is (very) low and when the plant material discolours during boiling. Boiling samples longer or in a higher concentration of NaOH did not improve the results.

The advantage of counting MS automatically is most evident in samples with a very high density. Counting of those samples by eye is almost impossible, because MS are too close to each other. Then, subsampling and diluting is another possibility. This would not solve the problem of aggregates containing many MS and may increase the variation. To diminish the variation by the IA method, (sub)sample sizes should be increased. This is only possible when

the surface of the filter paper, and as a consequence the surface scanned by IA are larger. To diminish the variation caused by subsampling it would be better to take more subsamples of one source. The number of subsamples will be a function of the accuracy required.

Selection on size is easily done by the computer programme and one of the possibilities to improve the results. Particles larger than MS of *V. dahliae* can be excluded (for example sclerotia of *Colletotrichum coccodes*). With the current IA method, it is impossible to discern between MS of *V. dahliae* and organisms which form structures with a similar size (for example MS of *V. tricorpus*). For that purpose other clearing techniques should be investigated. Specific staining could be a tool to overcome the problem with both the polluting non-biotic particles and other organisms. For now, the IA method should not be used for samples polluted with other dark particles, or should be used in combination with plating methods.

Because in potato material from Source 1 the correlation between IA counts and counts by eye was very good, for that purpose the IA method should be preferred. With IA the capacity is much higher and counting is less time consuming. Counting MS of *V. dahliae* by image analysis requires more research before it can be given a broader application.

CHAPTER 4

FORMATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE* ON CROPS

Verticillium wilt often included crop rotation with cereals. Wilhelm (1955), however, showed that *V. dahliae* was present in soil even after eight years of cropping with cereals or pasture.

V. dahliae can colonise plant roots (Lacy & Horner 1966; Evans & Gleeson 1973; Malik & Milton 1980) and many authors have observed formation of microsclerotia (MS) on roots, e.g. of cereals (Harisson & Isaac, 1969). Levy and Isaac (1976) observed more extensive growth and MS were more numerous on the barley roots than on pea roots.

Krikun and Bernier (1987) isolated *V. dahliae* from leaf tissues of infected wheat, barley, oats, pea, field bean, and rape plants, and found MS in the roots of all of these crops. Symptoms and effects on yield were observed in wheat, barley, and oats, but not in pea, field bean and rape. However, Hoekstra (1989) obtained substantial evidence for reduced yield of field bean in soil infested with *V. dahliae*. Malik and Milton (1980) inoculated onion, tulip, wheat and barley plants with *V. dahliae* by root dipping or by growing the plants in artificially infested soil. During growth the treated plants remained indistinguishable from those in the non-infested control treatments, even though MS formed in the roots of the four plant species. For studies on the effect of cropping sequences on the *V. dahliae* population in soil, it is important to know to what extent the subterranean debris of each crop in a rotation contribute to the soil inoculum density.

The removal or a treatment of infected plant debris might be a useful sanitation measure. It is feasible to remove above ground plant parts, but stubble removal is more difficult, because an extra soil tillage operation is required. It is virtually impossible to remove all root debris from the soil. The ratio between the formation of MS on aerial parts, the stubble and the root system is important when assessing the effects of sanitation measures. Ben-Yephet and Szmulewich (1985) and Mol and Scholte (1995a) quantified the formation of MS in the aerial and subterranean parts of different potato cultivars in ground plant material. They found that a substantial proportion of the MS on a potato plant is formed on the subterranean parts.

In the study described here, the MS formation on aerial and subterranean parts of different mono- and dicotyledonous plant species and cultivars was quantified. To ascertain whether the method of infestation affects the infection and colonisation of plant parts by *V. dahliae*, the MS formation was assessed after the plants had been infected by root dipping or by being grown in infested soil.

Materials and methods

Experiment 1

In 1992, nine crops were grown: potato (*Solanum tuberosum* cvs Element and Mirka; the former a susceptible and sensitive cultivar, and the latter a rather resistant and tolerant cultivar (Scholte & s'Jacob, 1990)), pea (*Pisum sativum* cv. Finale), sugar beet (*Beta vulgaris* cv. Univers), onion (*Allium cepa* cv. Jumbo), flax (*Linum usitatissimum* cv. Viking), spring barley (*Hordeum vulgare* cv. Prisma), field bean (*Vicia faba* cv. Victor), and spring wheat (*Triticum aestivum* cv. Minaret). On 5 May, rooted sprouts of potato or plantlets of the other crops were inoculated by root dipping and planted in 20-l pots in the open air under natural daylength and temperature (air temperatures from 5 May - 1 October 1993: mean 16.8°C, mean minimum 11.7°C, mean maximum 21.9°C). The pots were dug into the field to prevent them becoming heated by solar radiation. Per pot (0.08 m^2) the plant densities were 2 for potato, 7 for pea, 1 for sugar beet, 7 for onion, 35 for flax, 15 for spring barley, 4 for field bean and 15 for spring wheat. The plants were watered by hand twice a day to avoid water stress. There were eight replications.

The following amounts of nutrients were applied per pot, apportioned over three applications from planting date to six weeks after planting: 1.8 g N, 0.5 g P, 2.7 g K, 0.2 g Mg, and 12 ml of a trace element solution containing 20 g $MnSO_4.1H_2O$, 30 g H_3BO_3, 5 g $ZnSO_4.7H_2O$, 1 g $CuSO_4.5H_2O$ and 1 g $Na_2MoO_4.2H_2O$ per litre of tap water.

Experiment 2

In 1993, the same crops as in Experiment 1 were grown, plus potato cv. Ostara (2 plants per pot), potato cv. Astarte (2 plants per pot), and spring rape (*Brassica napus* cv. Petranova; 4 plants per pot). Plantlets of each crop were planted on 28 March and grown in 20-l pots in the open air under natural daylength and temperature (air temperatures from 28 March - 1 October 1993: mean 13.8°C, mean minimum 8.9°C, mean maximum 18.7°C). Each pot was wrapped in insulating foil to prevent it from being heated by solar radiation. The plants were watered by hand twice a day to avoid water stress. There were six replications.

The plants were infested by root dipping or by mixing MS with the soil in which they were planted. Each experimental unit with soil mixed with MS comprised two pots: one for harvesting mature aerial plant parts and one for harvesting the roots at an early stage of growth to recover maximum amounts of roots. The soil in each pot was fertilised with the same amount of nutrients as in Experiment 1, apportioned over three applications from planting date to six weeks after planting.

Infection of plants by root dipping

An isolate of *V. dahliae* was obtained from microsclerotia naturally produced on potato cv. Bintje stems in a commercial field of the Department of Agronomy in the autumn of 1990. Pure cultures were grown on potato dextrose agar slants at a temperature of ca. 22°C for two weeks.

Direct infestation of plants by the root dipping method was used to maximise the probability of infecting the plants. Plant seedlings or pre-rooted potato sprouts were infested by immersing their roots in a suspension of blended pure cultures of *V. dahliae* and were then planted in pots filled with a mixture of clay soil and potting compost (ratio 1:1 by volume).

Infection of plants by adding inoculum to soil

In autumn 1990, potato stems infested with microsclerotia of *V. dahliae* were collected from a commercial field of the Department of Agronomy with a history of growing potato. Stems were inspected and rejected if the sclerotia of other fungi were found. The selected stems were ground. The number of MS g^{-1} plant material was determined by image analysis (Mol & Meijer, 1995). In the experiments, the plants were grown in a mixture of a clay soil and potting compost (ratio 1:1 by volume) thoroughly mixed with MS to a final density of 30 MS per ml soil. To avoid infection at sites in the roots that had been damaged when the plants had been transplanted from the seedbed to the pots, a small hole was made in the soil in the centre of each pot and filled with non-infested soil, and the seedlings or pre-rooted potato sprouts were planted in this.

Collection of plant material

While the plants were growing, the senescent aerial plant parts were collected twice a week. The remaining aerial parts of potato, pea, flax, spring barley, field bean, spring wheat, and spring rape were harvested when the plants were mature. Aerial parts, stubble and roots were collected separately. Stubble was defined as all subterranean plant parts excluding roots and tubers but including an aerial stem base of ca 1 cm. The flax stems were harvested without separating the stubble from the stems. For the other maturely harvested crops, the aerial parts were removed 2-3 cm above the soil surface, and the stubble with the subterranean stem parts was harvested separately and washed in tap-water. Onion plants were harvested when the foliage had collapsed as a consequence of the softening of the pseudostems and when leaves started to die. As in commercial harvest, the leaves and bulbs were severed from the roots and left on the soil surface until the leaves had desiccated. Then the leaves were separated from the bulb. For sugar beet, the harvest of the leaves was in the first week of October, at the plant stage in which the sugar beet crop could be harvested commercially. The leaves and the epicotyl

of sugar beet were then chopped. Since MS are formed during senescence of the host tissue, a sample of the plant material from each sugar beet pot was kept in a permeable nylon bag for four weeks on the soil surface.

Roots in Experiment 2 were harvested when the maximum root density was expected. For barley, wheat, field bean, pea, rape and flax this was at the onset of flowering, for onion at the onset of bulbing, for potato when tuber bulking started, and for sugar beet in the first half of August. For each pot, the roots were washed with tap water, and were kept for four weeks in permeable nylon bags covered with the soil they had been grown in.

After harvest and incubation, all samples were air-dried, weighed, ground, and analysed for the number of MS.

Counting the microsclerotia

In both experiments the number of MS in ground plant material was directly counted 'by eye' as described by Mol and Meijer (1995). They estimated the number of MS by counting the number of black particles after boiling small samples of ground plant material suspended in 1 M sodium hydroxide.

Plant material of each sample was plated separately so that an independent test could be done for the presence of *V. dahliae*. A modified ethanol agar medium of Nadakavukaren and Horner (1959) was used. Water agar (20 g l^{-1}) was autoclaved for 20 min at 120°C, cooled down to ca 50°C, and kept at 45°C in a water bath. Just before pouring, 5 ml ethanol 96% and 50 mg chloro-oxytetracycline were added per l agar. After gently shaking, 60 plates l^{-1} were poured. Per sample, 15 mg ground plant material was spread over two plates. Uniform distribution was obtained by spreading this together with 0.7 ml 0.1% autoclaved water-agar over the surface of the medium. The plates were incubated for 3-4 weeks in a dark room at 23°C and a relative humidity of ca 90%. The number of colony forming units (CFU) was counted under a stereo dissecting microscope (magnification 12x).

Results

In the plants inoculated by root dipping, large differences in MS densities were found between the experiments and among crops (Table 1). In both experiments, the highest counts were recorded for potato cv. Element. The MS densities in potato cv. Mirka, pea, flax, and barley were higher in Experiment 2 than in Experiment 1, whereas for sugar beet the opposite was found. In Experiment 2, the MS density in the four potato cultivars decreased statistically

significantly from 'Element' via 'Ostara' and 'Astarte' to 'Mirka'. Flax, barley, and pea had densities more or less similar to those of 'Mirka'. The lowest numbers were found on field bean, wheat, onion, sugar beet, and rape.

The total number of MS per pot after infection by root dipping was calculated from the dry matter yield and the MS densities in the plant material (Table 1). In Experiment 1, potato cv. Element had by far the highest production, followed by cv. Mirka, sugar beet, field bean, wheat, pea, barley, flax and onion, in that descending order. In Experiment 2 potato cv. Element and flax had the highest production, followed by barley, potato cvs. Mirka, Ostara, and Astarte, field bean, pea, wheat, rape, sugar beet, and onion. Whereas in Experiment 2 the dry matter yields of the potato cultivars were not very different, there was a large difference in the dry matter yield between potato cvs Element and Mirka in Experiment 1. In most crops the dry matter yield was higher in Experiment 1 than in Experiment 2, causing higher MS yields per pot. In all samples, colonies of *V. dahliae* were found on the plates.

In the plants grown in infested soil, the MS density in the aerial parts and the stubble was the highest for the four potato cultivars (Table 2). The potato cultivars Element and Ostara had a higher MS density in the aerial parts than 'Astarte' and 'Mirka'. The MS density in the aerial parts of the other crops was much lower; the differences among crops and potato cultivars were much smaller in the stubble and root tissue than in the aerial parts. The number of MS counted in root tissue was low for all crops. In the stubble and the root very few CFU mg^{-1} were counted after plating the material. No *V. dahliae* colonies were found in the stubble of field bean and barley.

The number of MS per pot was estimated on the basis of the dry matter yield and the MS densities of the aerial parts, the stubble, and the roots (Table 3). In the aerial parts the highest production was found in flax. Among the potato cultivars, 'Element' had a significantly higher production per pot than 'Mirka', whereas 'Ostara' and 'Astarte' were intermediate. The other crops did not differ significantly and their levels were low. Differences in the number of MS per pot were much smaller in the stubble than in the aerial parts. Potato 'Astarte' gave the highest counts, followed by potato 'Element', 'Mirka' and 'Ostara', barley, wheat and rape, whereas field bean and pea showed the lowest numbers. Per pot, barley and flax showed the highest levels of MS in the roots followed by field bean, rape, wheat, pea, and potato 'Element'. Relatively low levels were found in the roots of onion, sugar beet, and potato 'Mirka', 'Ostara' and 'Astarte'. The total number of MS per pot was the highest for flax, followed by the four potato cultivars. The other crops showed lower total levels and did not differ mutually.

Table 1. Number of microsclerotia (MS) in aerial plant material (per mg and per pot) of 12 crops inoculated by root dipping. Experiments 1 and 2.

Crop	(MS.mg^{-1})[a]		(MS per pot x10^3)[a]	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Potato 'Element'	159.3 a	121.1 a	5506 a	2372 a
Potato 'Ostara'[b]	-	59.8 b	-	953 c
Potato 'Astarte'[b]	-	39.2 c	-	799 cd
Potato 'Mirka'	7.5 bc	21.6 de	1791 b	458 de
Field bean	4.7 c	5.9 e	992 c	474 de
Pea	5.7 bc	16.5 de	401 cd	712 cd
Flax	2.9 c	27.9 cd	216 cd	2032 a
Sugar beet[c]	15.7 b	3.8 e	1800 b	95 f
Onion	7.0 bc	3.9 e	93 d	57 f
Barley	4.6 c	24.9 de	382 cd	1589 b
Wheat	2.9 c	5.0 e	504 cd	460 de
Rape[b]	-	2.4 e	-	182 ef

[a]Different letters indicate significant differences between the treatments based on LSD values (P=0.05).
[b]Not present in Experiment 1.
[c]Values are very probably overestimated because plant particles turned dark during preparation of samples.

Table 2. Microsclerotial density in aerial plant material, stubble and roots of 12 crops grown in infested soil and the correlation with plating on semi-selective medium. Experiment 2.

Crop	Microsclerotial formation per mg plant material					
	Aerial[a]		Stubble[a]		Root[a]	
Potato 'Element'	91.2 a	+++	20.2 b	++	8.9 a	+
Potato 'Ostara'	83.3 a	+++	29.1 a	+	5.8 abc	+
Potato 'Astarte'	55.7 b	+++	20.2 b	++	2.5 cd	+
Potato 'Mirka'	47.3 b	++	11.2 c	+	5.7 abc	+
Field bean	2.8 c	+	1.2 d	0	1.9 cd	+
Pea	3.3 c	+++	3.2 d	+	2.8 cd	+
Flax	7.6 c	+++	-[b]		6.4 ab	+
Sugar beet[c]	2.8 c	+	-[b]		1.1 d	+
Onion	2.9 c	+	-[b]		3.6 bcd	+
Barley	2.2 c	+++	3.4 d	0	5.3 bc	+
Wheat	1.7 c	+++	2.8 d	+	3.6 bcd	+
Rape	1.6 c	++	3.9 d	++	2.6 cd	+

0= no colony forming units (cfu), + = 1-25% cfu, ++ = 26-50% cfu, +++ = 50% cfu-all relative to the number counted by eye.
[a]Different letters indicate significant differences between the treatments based on LSD values (P=0.05).
[b]No stubble left due to the harvest methods used.
[c]Values are very probably overestimated because plant particles turned dark during the preparation of the samples.

Table 3. Number of microsclerotia per pot formed on aerial plant parts, stubble or roots of 12 crops, counted by eye, after growing the plants in infested soil. Experiment 2.

Crop	Microsclerotia per pot (thousands)[a]				Rel.[b]
	Aerial	Stubble	Root	Total	
Potato 'Element'	1772 b	49.9 b	26.3 bcde	1850 b	100
Potato 'Ostara'	1452 bc	47.6 b	8.0 de	1509 bc	82
Potato 'Astarte'	1245 bc	92.2 a	5.5 e	1343 bc	73
Potato 'Mirka'	974 c	26.4 bc	13.5 cde	1014 c	55
Field bean	222 d	9.5 c	46.6 b	278 d	15
Pea	131 d	8.8 c	38.6 bcd	178 d	10
Flax	2447 a	-[c]	100.8 a	2547 a	138
Sugar beet[d]	222 d	-[c]	9.0 de	231 d	12
Onion	35 d	-[c]	14.1 bcde	49 d	3
Barley	192 d	34.2 b	117.6 a	343 d	19
Wheat	107 d	26.2 bc	40.6 bcde	174 d	9
Rape	139 d	48.3 b	43.8 bc	231 d	12

[a]Different letters indicate significant differences between the treatments based on LSD values (P=0.05).

[b]Relative differences calculated from the total production.

[c]No stubble left with the harvest methods used.

[c]Values are very probably overestimated because plant particles turned dark during the preparation of the samples.

Table 4. Yields of harvestable organs and formation of microsclerotia on aerial debris per pot, after infecting the plants by root dipping or by infestation of the soil. Experiment 2.

Crop		Yield (g dry matter per pot)		Microsclerotia per pot ($x10^3$)	
		Root dipping	Soil infestation	Root dipping	Soil infestation
Potato 'Element'	(tuber)	173.6 **	194.7	2372 *	1772
Potato 'Ostara'	(tuber)	164.2	168.2	953 *	1452
Potato 'Astarte'	(tuber)	205.8 **	229.7	799	1245
Potato 'Mirka'	(tuber)	227.5	225.7	458 *	974
Field bean	(seed)	142.9	144.9	474	222
Pea	(seed)	74.4	67.7	712 *	131
Flax	(haulm)	73.2 **	139.0	2032	2447
Flax	(seed)	17.4 **	36.9		
Sugar beet[b]	(beet)	237.5 **	260.7	95	222
Onion[a]		-	-	57	35
Barley	(seed)	85.5 *	103.7	1589 **	192
Wheat	(seed)	90.6	96.2	460	107
Rape[a]		-	-	182	139

* and ** Values for yield of dry matter and microsclerotia with root dipping differ significantly from those with soil infestation at $P<0.05$ and $P<0.01$, respectively.

[a]No yields were measured for onion and rape.

[b]Values are very probably overestimated because plant particles turned dark during the preparation of the samples.

The dry matter yield of harvestable organs was lower in potato 'Element' and 'Astarte', flax (seed and stem), sugar beet and barley from the root dipping treatment compared with the soil infestation treatment (Table 4). Root dipping resulted in higher levels of MS for potato 'Element', pea and barley. The difference with soil infestation was particularly large in barley. Potato 'Ostara', 'Astarte' and 'Mirka' showed statistically significantly higher levels of MS in the soil infestation treatment than in the root dipping treatment.

Discussion

Methodological problems

Between the two experiments there were large differences in MS densities and total numbers of MS for some crops. This might partly be attributable to different environmental conditions in the two years, but it is unlikely that this is the only reason. Large variation in MS formation in potato between experiments has been found previously, even in experiments carried out under controlled conditions in greenhouses (Mol & Scholte, 1995a, b). Moreover, in my two experiments, the differences were not similar for all crops. A difference in the time of harvest could be one of the factors.

Although colonies of *V. dahliae* were found in almost all samples after plating, there were large differences compared with the numbers counted by eye. There are at least two reasons for this. First, there is at least one MS when a CFU is counted, but there could easily be a cluster of many more MS, producing only one colony. Secondly, the germination of MS in the ground plant material could be poor, perhaps because of damage by grinding or other reasons. Also, the presence of plant tissue on the plates could have an effect: Malik and Milton (1980) found that germination of MS in tulip roots was rare when the MS were incubated in association with root tissue, but the MS germinated readily when dissected from the roots. In the current experiments, antagonistic organisms might have played a role in the low recovery after plating. This could have been a major factor in samples that were kept for some time in the soil or on the root surface. Therefore, counting by eye with a verification for *V. dahliae* by plating seems to be the best method for quantifying MS in different crops. Another explanation for differences between the two methods is that other black particles in the samples might inadvertently be counted by eye as microsclerotia of *V. dahliae*.

Sugar beet caused the largest problems in counting MS, because many particles in the sample turned dark after boiling in sodium hydroxide. Therefore, the levels counted in the

current experiments are probably overestimated. After plating, colonies of *V. dahliae* were counted. This shows that there is at least some reproduction of MS on sugar beet.

Comparison of infection methods

In plants infected by root dipping the MS density and MS yield tended to be higher than in plants infected by soil infestation. Exceptions were the potato cvs Ostara, Astarte and Mirka, and flax. A possible explanation is that the plants of the cvs Ostara and Mirka ('Astarte' showed the same tendency) were less susceptible in the early growth stage at which the roots were dipped. Their new roots grew into uninfested soil. In flax the yield was much lower after root dipping than after soil infestation, suggesting that the availability of plant tissue limits MS formation after root dipping. The dry matter yield reduction and the higher MS yield of barley after root dipping are remarkable. They confirm the findings of Mathre (1989), that wounding of barley roots facilitates infection by *V. dahliae*.

In field bean there was no yield reduction by root dipping and a very low production of MS, even though this crop is known as a good host (Hoekstra, 1989). In a field experiment Mol et al. (1995a) found an effect of the origin of the *V. dahliae* isolate on the yield and on the MS formation. In the current experiments, field bean may not have been sensitive or susceptible to the isolate used. Krikun and Bernier (1987) found that the colonisation of the aerial parts of gramineous crops also depended on the isolate used.

Differences among crops

The number of MS in the aerial parts was highest in potato and flax, indicating that these crops, which are known to be hosts of *V. dahliae*, were colonised systematically. In potato, the high numbers of MS were mainly caused by high MS densities, whereas in flax the high aerial dry matter yield was very important too. The higher MS formation on potato 'Element' than on 'Mirka' is consistent with previous results (Mol & Scholte, 1995a). The other crops were infected, but did not show a high density of MS in the aerial plant material.

Implications

Crops that have a high MS yield formed most of the MS in the aerial debris, which is not removed from the field with current cultivation practices. Research needs to be done on how removing the aerial debris after harvest affects the inoculum density in the soil. This effect cannot be estimated from the data obtained in the experiments described above, because it will interact with other factors in the field (e.g. the rate MS are released from plant debris, mortality rate of MS). From rotation experiments with *V. dahliae* it is difficult to conclude that some

crops are bad hosts. In a micro-plot experiment (Mol et al., 1995a), no decrease in the soil inoculum density of *V. dahliae* was found after two years of cropping with potato, pea, sugar beet, onion, flax, spring barley and field bean. This does not necessarily imply a long persistence in the soil, but might also point to a balance between formation of new MS and mortality.

Flax might contribute significantly to soil infestation if straw is left in the field after harvest. This is not common practice for fibre flax, for two main reasons: the straw is often harvested, and any straw left in the field hampers further cultivation practices. The debris of the other crops is usually left in the field. Once a good method for reducing the MS production in potato has been developed, attention should be paid to the reproduction in the other crops in the potato-based rotations too. So many MS are produced in wheat and barley that both the aerial and the subterranean parts can maintain a significant population in the soil. Since the recovery of the MS on the plates was very low, it would be worthwhile investigating the effect of sanitation measures on the soil inoculum density in these crops too.

Due to the large variation between experiments and the existence of some degree of host specificity (Mol et al., 1995a), the values obtained are not the maximum potential values for the crops grown. The inoculum density used was not very high, but because root growth in pots is abnormal, the roots in these experiments may have been more susceptible to infection than the roots of plants in the field. The large differences in MS formation among potato cultivars implies that in a rotation, the choice of potato cultivar is an important factor. Other authors have also found large differences between potato cultivars: Slattery (1981), Davis et al. (1983) and Mol and Scholte (1995a).

The results presented above cannot be considered to be conclusive for all field situations, but they can serve as a base for modelling at crop and cropping system levels for different crops, grown under comparable conditions.

SECTION 4.2

FORMATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE* ON VARIOUS PLANT PARTS OF TWO POTATO CULTIVARS

L. Mol and K. Scholte

Summary

Potato plants of cvs Element and Mirka were artificially infected with *V. dahliae* in two greenhouse experiments. Leaf blade, petiole, aerial stem, subterranean stem, stolon and root mass were separately harvested both when the canopy was still green and at maturity. After 4 weeks incubation, the plant tissue was air-dried and the numbers of microsclerotia per mg tissue and per plant were determined.

The highest numbers of microsclerotia were observed in the haulm when harvest took place at maturity. Cultivar Element yielded significantly more microsclerotia in the haulm than cv. Mirka, whilst there were no cultivar differences in microsclerotial production on subterranean parts. The petiole and the aerial stem contributed most to the total microsclerotial production, whereas roots were much more important for formation of microsclerotia than stolons.

Introduction

Formation of microsclerotia (MS) on plant debris is the most important survival mechanism of *Verticillium dahliae* Kleb. When infected plants senesce, the fungus leaves the xylem, readily permeates the surrounding tissues, and MS are produced in large quantities (Schnathorst, 1981). There is little evidence of other than transient increases in inoculum from other sources than plant debris (Powelson, 1970).

Slattery (1981) and Davis et al. (1983) reported large differences between cultivars in the number of MS.g^{-1} potato stem. Davis et al. (1983) found a sharp increase in MS density when harvest was delayed. However, they could not estimate the total MS production because they did not measure the dry mass of the stem.

Ben-Yephet and Szmulewich (1985) quantified the production of MS in the aerial and subterranean parts of two potato cultivars planted in autumn and spring by direct counts of MS in ground plant material. They reported a 100-fold greater MS production in the autumn crop, and attributed this difference to the dry hot weather during the senescence of the spring crop. In spring, the aerial and subterranean parts produced an equal number of MS, but in autumn a 20-40 times higher production was observed in the aerial parts than in the subterranean parts. This difference was mainly caused by the 10-times greater dry matter production in the aerial parts compared to the subterranean parts.

The most direct way of controlling *V. dahliae* is to interfere with the production and dispersion of the MS, e.g. through haulm treatments. For an accurate assessment of the effect of such treatments on the formation of MS, the MS density on various plant organs, the dry weight ratio of these organs, and the total number of MS per plant organ and per plant are needed. If most MS are formed on the aerial plant parts, a haulm treatment could affect the increase in inoculum potential of *V. dahliae*. This paper describes two greenhouse experiments in which MS production was measured in different parts of two potato cultivars at two harvest dates.

Materials and methods

An isolate of *V. dahliae* was obtained from microsclerotia naturally produced on potato stems (cv. Bintje) in a commercial field of the Department of Agronomy in the autumn of 1990. Pure cultures were grown on potato dextrose agar.

Plants of potato (*Solanum tuberosum* L.) cvs Element and Mirka, respectively susceptible plus sensitive and resistant plus tolerant to *V. dahliae* (Scholte & s'Jacob, 1990), were infected by immersing two detached rooted sprouts of each cultivar in a suspension of blended pure cultures of *V. dahliae*. The sprouts were planted in quartz sand in a 10 l pot on 28 May 1991 and placed in a greenhouse under natural daylength and a temperature of 22°C (day) and 15°C (night) with a thermoperiod of 14 h (Exp. 1). Both cultivars belong to an intermediate maturity class. The following quantities of nutrients were applied per pot, apportioned over 11 applications from the planting date to 10 weeks after planting: 8.6 g N, 2.2 g P, 12.6 g K, 1.0 g Mg, and 56 ml of a trace element solution containing 20 g $MnSO_4.1H_2O$, 30 g H_3BO_3, 5 g $ZnSO_4.7H_2O$, 1 g $CuSO_4.5H_2O$ and 1 g $Na_2MoO_4.2H_2O$ per litre of water.

The first harvest (D1) was 72 days after planting, at which time wilt symptoms were visible and lower leaves had started to senesce. The second harvest (D2) was 113 days after planting, when the leaves were senescing but the stems were still green.

At each harvest, leaf blades, petioles, aerial stem parts, subterranean stem parts, stolons, roots and tubers were separated, and the fresh yields of each part were determined. Except for the tubers, all plant parts were cut into pieces of ± 5 cm. A sample of each part was incubated in a nylon bag for 4 week. The aerial parts were incubated on the soil surface, and the subterranean parts were covered with a layer of 10 cm non-sterilized sandy soil (pH= 5.5; 2.7% organic matter). From another sub-sample of each plant part the dry matter content was determined immediately after each harvest. After incubation, the samples were air-dried and the loss of dry mass during the 4-weeks period was calculated. Except for the tubers the samples were ground, and stored until the MS were counted. The experiment was laid out as a randomised complete block design with six replications.

In 1992 the experiment was planted on 31 July (Exp. 2) and repeated under similar conditions. The following quantities of nutrients were applied per pot, apportioned over four applications from planting to 10 weeks after planting: 2.5 g N, 0.6 g P, 3.6 g K, 0.3 g Mg, and 16 ml of the trace element solution also used in Exp. 1. The first and the second harvests were on 53 (D1) and 92 (D2) days after planting. In Exp. 2 the tubers were not harvested. The experiment also had six replications.

In Exp. 1, the MS were counted by eye as described by Mol and Meijer (1995), and in Exp. 2 they were counted using an image analysis system also as described by Mol and Meijer (1995). These methods estimate the number of MS from the number of black particles present after boiling small samples of ground plant material in a 1 M sodium hydroxide solution. In Exp. 1, the MS on the surface of the tubers were counted using a binocular microscope (magnification 24x).

Results

Density of microsclerotia

In Exp. 1, the leaf blades, petioles, and aerial and subterranean stems of cv. Element yielded consistently more MS.mg^{-1} plant tissue than those of Mirka (Table 1). Differences were largest on D2. In Exp. 2 cultivar differences in MS densities were only found in the petioles on D2. Although not significant, on D2 there was a trend in both experiments for cv. Element to have more MS in subterranean stem parts and stolons than cv. Mirka, but this trend was not

observed for the roots. In both experiments the MS density in petiole and aerial stem was much higher on D2 than on D1. For all plant parts, the MS densities measured in Exp. 2 were higher than in Exp. 1. Of all the tubers investigated only two contained MS.

Plant yield

Total plant weight (excluding tubers) was much higher in Exp. 1 (Table 2). In Exp. 1 the plants, as well as their separate parts, had a much higher dry matter yield on D2 than on D1. In Exp. 2 no yield differences were observed between the harvest dates. In Exp. 1 the total yield and the leaf blade, aerial stem, stolon, and root yields of cv. Mirka were higher than those of cv. Element. In Exp. 2 the aerial stem yield was lower and the stolon and root yields were higher in 'Mirka' than in 'Element'.

Production of microsclerotia

The production of microsclerotia per plant showed large differences among plant parts and between cultivars, harvest dates and experiments (Table 3). There were significantly more MS counted on D2 than on D1, both for the total number of MS per plant and for the aerial parts (leaf blade, petiole and aerial stem) separately.

In Exp. 1 the numbers of MS in the subterranean parts were higher on D2, whereas in Exp. 2 the numbers were higher on D1. Among the subterranean parts the roots contributed most to the total number of MS. However, most MS occurred in the aerial parts. On D2 the stems had the highest number of MS among the aerial parts, but on D1 the leaf blades and petioles also contributed substantially to the total number. In the aerial parts, and as a consequence in the plant as a whole, the number of MS was much higher in cv. Element than in Mirka. The proportion of the MS formed in the aerial parts was 83-97% and 56-92% for cvs Element and Mirka, respectively. The ratios between D1 and D2 for the total numbers of MS per plant were the same for both cultivars; 'Element' and 'Mirka' had values of 12-16% and 32-36% for Expts 1 and 2 respectively.

Table 1. Density of microsclerotia (numbers per mg) in air-dried plant material on various plant parts of the potato cvs Element and Mirka after harvesting a green (D1) or a mature crop (D2). Data from two experiments.

Plant material	Exp. 1					Exp. 2				
	D1		D2		Sign.[a]	D1		D2		Sign.[a]
	'Element'	'Mirka'	'Element'	'Mirka'		'Element'	'Mirka'	'Element'	'Mirka'	
Leaf blade	2.0	1.2	6.1	1.6	C	27.8	25.9	42.5	43.8	D
Petiole[b]	10.4 b	2.0 b	32.5 a	2.4 b	C*D	19.7 b	12.7 b	197.0 a	41.3 b	C*D
Aerial stem[b]	17.1 b	0.6 c	46.2 a	5.1 bc	C*D	17.0	9.0	149.0	124.0	D
Subterranean stem[b]	3.8 b	2.4 b	15.4 a	5.8 b	C*D	41.8	48.7	36.8	24.5	
Stolon	3.4	8.3	8.2	4.8		37.2	27.3	58.0	23.2	
Root	6.5	4.5	10.9	6.5	C,D	25.8	18.8	28.0	32.8	

[a]C, D and C*D indicate a significant difference ($P<0.05$) within a plant organ between cultivars or harvest dates or a significant interaction, respectively.

[b]Different letters indicate significant differences ($P<0.05$) between the two cultivars and harvest dates, within a plant organ of one experiment.

Table 2. Dry matter yield ($g.pot^{-1}$) of the total plant and various plant parts of the potato cvs Element and Mirka after harvesting a green (D1) or a mature crop (D2). Data from two experiments.

Plant part	Exp. 1					Exp. 2				
	D1		D2		Sign.[a]	D1		D2		Sign.[a]
	'Element'	'Mirka'	'Element'	'Mirka'		'Element'	'Mirka'	'Element'	'Mirka'	
Total plant[c]	31.4	40.2	65.9	95.3	C,D	25.5	19.0	18.1	20.4	
Leaf blade	15.0	16.7	21.0	27.5	D	11.2	8.6	9.0	9.7	
Petiole	3.9	4.0	7.8	9.6	D	2.6	2.1	2.2	2.8	
Aerial stem	6.4	13.3	27.2	39.2	C,D	8.4	4.3	5.4	4.7	C
Subterranean stem	1.6	1.3	2.7	1.9	D	1.0	0.6	0.5	0.6	
Stolon[b]	0.6 b	0.5 b	0.9 b	3.1 a	C*D	0.4	0.6	0.1	0.6	C
Root[b]	3.9 b	4.4 b	6.3 b	14.0 a	C*D	1.9	2.8	0.9	2.0	C

[a]C, D and C*D indicate a significant difference ($P<0.05$) within a plant organ between cultivars or harvest dates or a significant interaction, respectively.

[b]Different letters indicate significant differences ($P<0.05$) between the two cultivars and harvest dates, within a plant organ of one experiment.

[c]Excluding tubers.

Table 3. Number of microsclerotia ($x10^{-3}$) per pot on various air-dried plant parts of the potato cvs Element and Mirka after harvesting a green (D1) or a mature crop (D2). Data from two experiments.

Plant material	Exp. 1					Exp. 2				
	D1		D2		Sign.[a]	D1		D2		Sign.[a]
	'Element'	'Mirka'	'Element'	'Mirka'		'Element'	'Mirka'	'Element'	'Mirka'	
Leaf blade	30	20	128	44	D	312	223	382	426	D
Petiole[b]	41 b	8 b	252 a	23 b	C*D	51 bc	27 c	440 a	117 b	C*D
Aerial stem[b]	110 b	8 b	1257 a	200 b	C*D	142	39	803	576	D
Subterranean stem[b]	6 b	3 b	42 a	11 b	C*D	42	28	20	14	D
Stolon	2	4	7	15	D	14	16	5	13	
Root[b]	25	20	69	91	D	48 ab	52 a	26 b	66 a	C*D
Sub-total aerial[b]	181 b	35 b	1635 a	266 b	C*D	505 c	289 c	1625 a	1119 b	C*D
Sub-total subterranean[c]	33	27	117	118	D	103 a	97 a	52 b	92 a	C*D
Total[b,c]	214 bc	62 c	1753 a	384 b	C*D	608 c	386 c	1676 a	1212 b	C*D

[a]C, D and C*D indicate a significant difference ($P<0.05$) within a plant organ between cultivars or harvest dates or a significant interaction, respectively.

[b]Different letters indicate significant differences ($P<0.05$) between the two cultivars and harvest dates, within a plant organ or group of organs of one experiment.

[c]Excluding tubers.

Discussion

Density of microsclerotia

The resistant cv. Mirka yielded significantly fewer MS in the stems and petioles than the susceptible cv. Element. Slattery (1981) and Davis et al. (1983) also found differences between cultivars in MS production. In Exp. 1, the MS density in the aerial stems of cv. Mirka showed a similar level to that found by Davis et al. (1983), who counted the number of colony forming units from air-dried ground potato stems from field grown plants of different cultivars. The MS density counted in stems in Exp. 2 was much higher than that of Davis et al. (1983). However, they only counted MS in immature plant material that did not show wilt symptoms at harvest. It is also not known if their cultivars had a resistance to *V. dahliae* similar to that of 'Element' and 'Mirka'. The MS densities in stem tissue counted in our experiments are in the same range as those found by Slattery (1981) on mature stems.

The differences between Expts 1 and 2 could be caused by the application of much larger amounts of nutrients to Exp. 1. Davis and Everson (1986) found a significant correlation

between N concentration in petioles and stem colonization with *V. dahliae* for cv. Russet Burbank, and also that the percentage of stems with Verticillium wilt symptoms decreased with increasing nitrogen fertilization. Davis et al. (1990) observed a significantly lower number of colony forming units per g haulm tissue with an increasing P-concentration in the soil.

Production of microsclerotia

Differences in the yield of MS between plant parts depend both on the MS density and the dry matter production. Although subterranean plant parts may yield considerable MS densities, the contribution of the subterranean parts to the total production was only 3-17% for 'Element' and 8-44% for 'Mirka'. The difference between the two cultivars was mainly caused by the higher root mass of 'Mirka'. The number of MS per plant in the subterranean parts was similar for both cultivars. Ben-Yephet and Szmulewich (1985) suggested that the contribution of the subterranean parts without roots to the total production was 0.25-0.5% for an autumn crop. This agrees with the results of our experiments if the contribution of the roots is ignored.

The total number of MS per plant on D2 agrees with the counts of Ben-Yephet and Szmulewich (1985) in the autumn crop. In our experiments there was a large difference between the immature harvest (D1) and the mature harvest (D2). This agrees with the results of Davis et al. (1983). Drying up of the haulm tissue limits the formation of MS (Ioannou et al., 1977c; Erwin et al., 1978). Moreover *V. dahliae* is a weak competitor for its food source (Ioannou et al., 1977a). When the crop is harvested more mature *V. dahliae* would already have started to grow to the outer part of the tissue and competitors would develop too late to prevent its reproduction. Another reason for the higher numbers at a later harvest could be the number of propagules in the stem tissue before harvest. In two mint species, Brandt et al. (1984) found a sharp increase in the number of propagules of *V. dahliae* with time.

Implementation in cropping systems

The two harvest dates correspond with the times at which the haulm is killed in The Netherlands for seed potatoes (D1) or ware potatoes (D2). Consequently, in fields where seed potatoes are grown the build-up of the MS population in the soil will be much lower compared to fields cropped with ware potatoes. Furthermore, it may be assumed that the effect of a potato crop on the build-up of soil infestation can be considerably reduced in a crop rotation in which the formation of MS in aerial plant parts or the spread of aerial plant debris into the soil is reduced by cultural practices.

CHAPTER 5

EFFECTS OF CROP SPECIES, CULTIVARS, AND TWO ISOLATES OF *VERTICILLIUM DAHLIAE* ON THE POPULATION OF MICRO-SCLEROTIA IN THE SOIL, AND CONSEQUENCES FOR CROP YIELD

CHAPTER 5

EFFECTS OF CROP SPECIES, CROP CULTIVARS AND ISOLATES OF *VERTICILLIUM DAHLIAE* ON THE POPULATION OF MICROSCLEROTIA IN THE SOIL, AND CONSEQUENCES FOR CROP YIELD

L. Mol, J.M. van Halteren, K. Scholte and P.C. Struik

Summary

In a micro-plot experiment the development of the inoculum density of *Verticillium dahliae* in soil was studied and consequences for yields of potato cvs Element, Ostara, Astarte, and Mirka; field bean; flax; pea; barley; sugar beet; and onion were evaluated. In May 1991, 75-litre containers were filled with sterile soil. Soil was infested with 2 or 200 microsclerotia.g^{-1} of a potato isolate or a field bean isolate of *V. dahliae*. The same crop species and cultivar was grown on the same plot in 1991 and 1992. For flax, a non-pulled crop was compared with a pulled crop. Fallow plots were included as a control. In 1993, potato cv. Element was grown on all plots. During three years the soils were sampled, and the soil inoculum densities were assessed by plating the soil on a semi-selective medium.

In 1991, yield differences between isolates were only significant for potato cv. Element (lower yield when infected with potato isolate), and potato cv. Astarte, field bean, and sugar beet (lower yield when infected with field bean isolate). In 1992, isolates and infestation levels did not affect the yields. In 1993, haulm dry matter yield of cv. Element was affected by the isolate, the inoculum density and the crop grown in the previous years. Plots previously cropped to potato cvs Element, Astarte and Mirka gave the lowest yields in 1993.

The inoculum densities of the low-level infested fallow plots increased until the end of the third year, whereas the inoculum densities of the high-level infested fallow plots stabilised after the second year. No decrease of inoculum density occurred under any crop species or cultivar grown. Increase of inoculum densities was significant for potato cvs Element, Ostara, Astarte, and field bean at the low initial soil infestation level. At the high initial soil infestation level the effect of crop species and cultivar on the inoculum densitiy depended on the isolate used. Potato cultivars were more susceptible to potato isolate than to field bean isolate and the opposite was

true for field bean. Removing flax culms from the field diminished the increase of the inoculum density in treatments infested with potato isolate at a high level.

In 1993, the concentration of microsclerotia in the haulm debris was positively related to the inoculum density in the soil, and the haulm yield was negatively related to the inoculum density in the soil.

Introduction

Since microsclerotia (MS) of *Verticillium dahliae* Kleb. can survive up to 14 years in the field under non-host croppings (Wilhelm, 1955) and because of the pathogen's broad host range (Woolliams, 1966), short crop rotations cannot successfully control *V. dahliae*. The use of non-hosts to lower the soil inoculum density (ID) has been suggested in the past (Schreiber & Green, 1963), but recent work of Mol (1995b) showed little effect of luring crops on ID. The exudates of such crops would stimulate the MS to germinate and exhaust the MS in this way. An important reason for the observed persistence of MS in soil can be the capability of *V. dahliae* to form colonies on plant roots regardless whether the crop is a host or a non-host (Lacy & Horner, 1966; Mol, 1995a).

Microsclerotia are formed in aerial and subterranean plant parts of many field crops (Mol, 1995a), but the contribution of the aerial parts is by far the largest (Mol & Scholte, 1995a). The release of MS from plant debris in the next year after incorporation of the debris in the soil can cause an increase in ID if the release is faster than the decrease of MS due to mortality (Ashworth et al., 1974; Huisman & Ashworth, 1976; Joaquim et al., 1988; Mol et al., 1995b).

Inconsistent results for the relationships between plant infection or yield loss and ID are reported by a number of researchers (Termorshuizen & Mol, 1995). Nicot & Rouse (1987) stated that two colony forming units (CFU).g^{-1} soil are sufficient for 70-100% infection of potato stems. Threshold levels for yield loss vary from 0.6 CFU.g^{-1} soil (Ben-Yephet & Szmulewich, 1985) to more than 46 CFU.g^{-1} soil (Davis & Everson, 1986). The major reasons for this wide range are: a) that *V. dahliae* is a weak competitor compared to other organisms in the rhizosphere (Brinkerhoff, 1969; Ioannou et al., 1977a), and b) the positive effect of higher temperatures on disease development and plant growth (Nnodu & Harrison, 1979). Therefore, the infection rate not only depends on the ID but also on interactions with soil micro-organisms and effects of abiotic factors.

Potato cultivars differ in extent of MS formation in plant tissue (Slattery, 1981; Ben-Yephet & Szmulewich, 1985; Mol & Scholte, 1995a), and also in sensitivity to *V. dahliae* (Scholte et

al., 1985). Differences in both resistance and sensitivity among different crop species and cultivars could be influenced by isolate specificity of *V. dahliae*. The virulence of an isolate is more related to the cropping history than to the crop the pathogen was isolated from (Tjamos, 1981).

In this paper results are reported from an experiment in which changes in ID were measured as influenced by cropping of ten crop species and cultivars during two subsequent years; crop yields are reported as well. Soil was initially infested in two densities with two isolates of *V. dahliae* obtained from soils with different cropping histories. In the third year a susceptible potato cultivar was grown allowing analysis of the effects of the crop species and cultivar grown in the previous two years and of the initial infestation level.

Materials and methods

Preparation of inoculum

Soil samples were taken from fields where either potato or field bean had been grown continuously for 14 years (P-soil) and for ten years (F-soil) respectively. Potato (*Solanum tuberosum* L., cv. Element) was planted in P-soil and field bean (*Vicia faba* L., cv. Alfred) in F-soil. From these plants *V. dahliae* isolates were obtained by plating stem segments on semi-selective pectate agar (Huisman & Ashworth, 1974) and pure cultures of *V. dahliae* established on potato dextrose agar. Two weeks later, the cultures were shaken with water and the suspension was used to inoculate pieces of green potato (potato isolate) or field bean (field bean isolate) stems previously sterilised by autoclave at 120°C for 20 minutes in Erlenmeyer flasks.

After two weeks of incubation at 23°C in the dark, large numbers of microsclerotia (MS) were present on the stem pieces. The stems of each plant species were air-dried and ground through a 1 mm sieve. Microsclerotia in subsamples of the ground material were quantified using a stereo dissecting microscope. The numbers of MS $gram^{-1}$ dry material were $9.5x10^5$ for potato isolate (PI) and $8.5x10^5$ for the field bean isolate (FI).

Micro-plots

A long-term factorial experiment with 240 micro-plots was laid out as a randomised complete block design with five replicates. Plastic containers of 75 l (60x40x32 cm) and holes in the bottom to allow drainage were buried in the centre of a 1.5x1.5 m plot on sandy soil in April 1991. A mixture of sand and pot soil (2:1 v/v; density ca 1.0 $g.cm^{-3}$) was pasteurised twice, kept for 2 months to have it recolonised by micro-organisms and artificially infested with PI or

FI by thoroughly mixing inoculum with soil in two densities: 2 and 200 MS g^{-1} air-dry soil for a low and a high level, respectively.

Both in 1991 and 1992, each micro-plot was cropped with the same crop. The following crop species and cultivars were grown: potato cvs Element, Mirka (susceptible plus sensitive and resistant plus tolerant to *V. dahliae*: Scholte & s'Jacob, 1990), Ostara and Astarte; field bean cv. Alfred; flax (*Linum usitatissimum* L., cv. Viking); pea (*Pisum sativum* L., cv. Finale); barley (*Hordeum vulgare* L., cv. Prisma); sugar beet (*Beta vulgaris* L., cv. Univers); and onion (*Allium cepa* L., cv. Jumbo). For flax two harvest methods were included: one method comprised removal of the straw together with the seed ('pulled flax'), and with the other method only the seed was harvested, and straw was returned to the plot ('non-pulled flax'). In 1991 and 1992, also control plots without a crop were included in the experiment ('fallow'). In 1993, all 240 plots were cropped with potato cv. Element.

Cultivation and crop measurements

In 1991, planting was in the first week of July, whereas planting in 1992 and 1993 was in April. All crops were also planted in the area of 1.5x1.5 m directly surrounding the micro-plots to prevent border effects. Plant densities were 3 plants.$plot^{-1}$ for sugar beet; 4 (single-stemmed plants) for potato; 8 for field bean; 18 for pea; 40 for onion; 60 for barley; and 480 for flax.

Soil fertility was analysed every year and the results were used to assess necessary nutrient supply. The pH-KCl of the soil was 5.8 and the organic matter content was 10.3%. Weeds were regularly removed from all plots and the whole field was irrigated when needed to avoid water stress.

In 1991 and 1992, at harvest, yields of aerial plant parts were assessed, and a sample was taken to assess the dry matter concentration. The aerial plant parts were returned to the plot where they had been grown. In 1991, onion bulbs, potato tubers and sugar beet roots were harvested. In 1992, harvestable organs were collected for all crops, except for linseed. Of all samples dry matter concentrations were determined. At the end of the season plant debris and soil were mixed together using a 160 l concrete mixer, and each micro-plot was refilled with its own soil.

In 1993 on July 30, August 6 and August 13, the greenness of the potato haulm was measured qualitatively on a scale from 1 (dead) to 10 (completely green). At harvest, the aerial plant parts and tubers were weighed and the dry matter concentrations determined. All remaining plant parts, except the roots, as well as the upper surface soil, were removed in October 1993 to prevent any contribution of this potato debris to the build-up of the soil

inoculum density of *V. dahliae*. In the haulm debris of each plot, the number of MS was determined by image analysis (Mol & Meijer, 1995).

Soil analysis on microsclerotia

All plots were sampled with a 2.0 cm diameter auger to a depth of 20 cm in March 1992, October 1992, March 1993, October 1993, and March 1994 to measure the MS population of *V. dahliae*. In October, the samples were taken before the aerial plant debris of the last crop was mixed through the soil. Each sample consisted of six cores and had a total weight of approximately 250 g air-dry soil. The samples were air-dried in the laboratory for two weeks and sieved through a 2.0 mm mesh.

For plating soil, a modified ethanol agar medium of Nadakavukaren and Horner (1959) was used. Just before pouring, per liter agar 5 ml ethanol 96% (vol/vol) and 50 mg chloro-oxytetracycline was added to water agar (20 $g.l^{-1}$) that had been autoclaved for 20 min at 120°C and cooled to 50°C.

A 20 g subsample was taken from each soil sample and added to 90 ml 0.1% agar solution to make a 100 ml suspension (the viscous 0.1% agar solution was used to ensure a better distribution in the sample during mixing) and from each soil suspension 0.5 ml was spread over each of five Petridishes. These were incubated at high humidity (90-95% r.h.) and at a temperature of 22°C in the dark. After three weeks the soil was washed from the medium by gently rubbing and numbers of colonies of *V. dahliae* were counted using a stereo dissecting microscope (magnification 12x).

Results

Crop yields in 1991 and 1992

Except for flax, all crop species and cultivars had a higher total dry matter yield in 1992 than in 1991. In 1991, the average crop yield on plots with a low infestation level was significantly higher than on plots with a high infestation level (data not shown). No significant interaction occurred between crop species and cultivar and infestation level.

In 1991, potato cv. Element had a 29% lower total dry matter yield in plots infested with potato isolate (PI) than on plots infested with field bean isolate (FI) (Table 1), whereas yields of cv. Astarte were 17% lower in FI-plots than in PI-plots. Yields of field bean and sugar beet were lower in FI-plots than in PI-plots (31% and 22%, respectively). In 1992, no significant differences in crop total dry matter yields occurred between the two isolates.

Table 1. Effect of isolates of *V. dahliae* (PI= potato isolate and FI= field bean isolate) on the dry matter yield of ten crop species and cultivars averaged over a low and a high infestation level.

Crop	Isolate	Dry matter yield (g per plot)		
		1991		1992
Potato 'Element'	PI	296		471
	FI	382	***	488
Potato 'Ostara'	PI	202		411
	FI	214		442
Potato 'Astarte'	PI	380		639
	FI	324	*	652
Potato 'Mirka'	PI	288		625
	FI	287		596
Field bean	PI	239		453
	FI	182	*	384
Flax	PI	227		191
	FI	230		213
Pea	PI	166		309
	FI	175		300
Barley	PI	148		200
	FI	123		207
Sugar beet	PI	361		682
	FI	282	**	682
Onion	PI	73		196
	FI	48		196

*, **, *** indicate significant differences between potato and field bean isolate infested treatments at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Inoculum density over time

The grand means for ID's found in March 1992 (D1), October 1992, March 1993, October 1993 and March 1994, were 5.6, 10.0, 13.5, 20.0 and 20.0 CFU.g^{-1} soil, respectively. The ID's of October 1992 and March 1993 were averaged for statistical analysis (D2), and also the ID's of October 1993 and March 1994 (D3).

In fallow plots with low initial infestation level, the ID's increased until the third year, whereas with high initial infestation level the ID's stabilised after the second year (Table 2). At any sampling date, there was no crop species or cultivar with a significantly lower ID than the fallow control.

Under all crop species and cultivars after three years, the ID's measured were higher for the treatments with the high initial infestation level than for the low initial level of infestation, except for potato cv. Element grown in FI-plots (32.2 vs. 39.2 CFU g^{-1} soil, respectively) and for field bean grown in PI-plots (10.2 vs. 12.6 CFU g^{-1} soil, respectively).

Table 2. Effect of crop species and cultivars and two different isolates (PI= potato isolate and FI= field bean isolate) on the number of colony forming units (cfu) per g soil at a low and a high initial infestation level on three dates (D1= spring 1992, D2= winter 1992-1993, and D3= winter 1993-1994).

Crop	Isolate	Infestation level (cfu per g soil)					
		Low			High		
		D1	D2	D3	D1	D2	D3
Fallow control	PI	1.2	2.8	7.4	8.8	25.8	22.8
	FI	0.0	1.6	3.6	5.2	17.6	17.2
Potato 'Element'	PI	3.6	19.6	45.0	23.6	50.6	76.6
	FI	4.0	6.2 ***	39.2	4.8 ***	13.8 ***	32.2 ***
Potato 'Ostara'	PI	1.2	6.8	30.4	16.4	42.6	67.2
	FI	0.8	6.0	28.2	12.4	20.4 ***	52.8 (*)
Potato 'Astarte'	PI	0.8	3.8	15.6	14.4	17.6	38.6
	FI	0.4	6.0	18.8	3.6 *	8.6	25.8 (*)
Potato 'Mirka'	PI	0.0	2.0	5.2	11.6	17.3	34.2
	FI	0.0	2.8	7.4	7.6	11.8 ***	14.4 **
Field bean	PI	0.8	1.0	12.6	5.2	7.6	10.2
	FI	0.4	3.8	14.8	10.1	33.6 ***	62.6 ***
Flax pulled	PI	0.4	1.0	4.6	17.2	19.6	20.0
	FI	0.0	3.2	4.6	5.2 *	14.6	15.4
Flax non-pulled	PI	0.0	1.6	3.8	20.0	30.8	36.4
	FI	0.0	2.2	4.2	7.2 *	11.6 **	16.4 *
Pea	PI	0.0	6.0	5.0	10.8	17.6	22.2
	FI	0.0	4.8	3.8	4.8	14.6	27.2
Barley	PI	1.6	5.2	4.0	11.6	24.4	15.6
	FI	0.0	4.8	3.2	4.4	12.6	15.2
Sugar beet	PI	0.0	1.2	2.0	18.8	14.2	18.4
	FI	0.4	0.8	4.8	11.2	12.9	14.6
Onion	PI	0.8	1.6	2.2	18.8	14.2	18.4
	FI	0.0	1.8	2.8	9.2 *	15.2	11.4

(*), *, **, *** indicate significant differences between potato and field bean isolates at $P<0.10$, $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Effects of crop species and cultivars and isolates at the high initial infestation level differed considerably from the effects at low initial infestation level. On D1 all ID's were smaller than the initial infestation level (2 and 200 MS.gram^{-1} air-dry soil for low and high levels, respectively), only the ID of potato cv. Element at low initial infestation level was higher but not significantly different. On D3 at low initial infestation level, plots grown with potato cvs Element, Ostara, or Astarte, or field bean had a significantly higher ID (mean for potato and

field bean isolates) than the fallow control. Cropping with potato cv. Element resulted in a higher ID than cropping with potato cv. Ostara. All other potato cultivar differences were significant as well. Values for field bean were similar to values for 'Astarte'. Inoculum levels found in plots grown with potato cv. Mirka, pulled and non-pulled flax, pea, barley, sugar beet and onion, did not differ from the fallow control. Effect of isolate was only found for potato cv. Element on D2 when a higher ID was measured for PI than for FI.

At the high initial infestation level, effects of crop species and cultivars interacted with those of isolates. All potato cultivars and non-pulled flax had a higher final ID in PI-plots than in FI-plots. In PI-plots, non-pulled flax showed a higher ID than pulled flax on all dates, but this effect was absent in FI-plots. The difference between pulling and non-pulling on PI-plots gradually increased over time from statistically not significant on D1, to significant on D2 ($P<0.10$) and D3 ($P<0.05$). Under field bean, ID of PI-plots did not increase; it even tended to be somewhat lower than the control, whereas ID of FI-plots increased strongly. Pea, barley, sugar beet and onion did not affect the ID at either the low or the high initial infestation level.

Effect of inoculum density and preceding crop on potato

The effect of the inoculum density and the preceding crop on potato cv. Element was analysed with an analysis of covariance. The mean of the inoculum density in March 1993 and October 1993 was used as the covariable. Effects of the initial infestation level were not significant in the analysis and included in the covariable.

On July 30 the PI-plots showed a lower greenness than the FI-plots (Table 3). Among the crop species and cultivars grown in the preceding two years differences only occurred between some potato cultivars and other crop species, but differences were small. There was a significant effect of the covariable, indicating that a higher ID gave a lower value for greenness. For the August 6 and 13 observations the same trend was observed.

Haulm dry matter yield was lower for PI than for FI treatments (Table 3). There was a significant crop x isolate interaction caused by the fallow control plots. In PI fallow control plots, the haulm dry matter yield was significantly lower than in FI fallow control plots, but the isolate did not affect haulm yield when a crop was grown in the preceding years. Potato cvs Element, Astarte and Mirka showed lowest haulm yields, whereas cv. 'Ostara' occupied an intermediate position among these three potato cultivars and the other crop species. An increasing ID resulted in a decreasing haulm dry weight. The potato cvs Element, Astarte and Mirka as a preceding crop gave a lower tuber dry matter yield than the other crop species or cultivar and the fallow control. There was no significant additional effect of the ID.

Table 3. Effect of soil infestation with a potato and a field bean isolate of *V. dahliae* followed by two years of cropping with different crop species and cultivars on the senescence, dry matter (DM) yield, concentration of microsclerotia (MS) in mature haulm and the relative number of MS on mature haulm formed per plot on the subsequent potato crop, and the contribution of the soil inoculum density (ID) in colony forming units (cfu) calculated by an analysis of covariance.

Crop in the two preceding years	Greenness on July 30[a]	Haulm DM[a] ($g.plot^{-1}$)	Tuber DM[a] ($g.plot^{-1}$)	MS formation[a] ($particles.mg^{-1}$)	Rel. $MS.plot^{-1}$ [a]
Fallow control	5.9 abc	17.2(PI)* 26.6(FI)	520 a	527 c	59 cd
Potato 'Element'	5.0 bcd	15.2 cd	388 b	1142 a	100 a
Potato 'Ostara'	5.5 abcd	17.8 bc	507 a	802 b	83 abc
Potato 'Astarte'	4.5 d	15.1 cd	390 b	1146 a	98 ab
Potato 'Mirka'	4.9 cd	12.3 d	360 b	1045 a	69 cd
Field bean	6.0 abc	19.8 ab	510 a	604 bc	64 cd
Flax pulled	6.1 ab	18.3 b	517 a	643 bc	65 cd
Flax non-pulled	6.4 a	19.8 ab	519 a	671 bc	75 abcd
Pea	6.5 a	22.0 a	539 a	604 bc	69 cd
Barley	5.8 abc	19.6 ab	514 a	599 bc	64 cd
Sugar beet	6.4 a	19.5 ab	533 a	704 bc	74 bcd
Onion	6.5 a	20.3 ab	530 a	513 c	54 d
Mean Potato Isolate	5.4 **	17.4 ***	481 n.s.	870 ***	100 ***
Field bean Isolate	6.2	19.5	490	630	75
Effect of ID (per $cfu.g^{-1}$(soil))	-0.031 ***	-0.064 **	- n.s.	5.8 ***	- n.s.

[a]Different letters indicate significant differences ($P<0.05$) between crops. (*),*,**, ***, indicate significant differences between potato and field bean isolate or a significant effect of ID at $P<0.10$, $P<0.05$, $P<0.01$, and $P<0.001$ respectively. n.s. = not significant.

The concentration of MS, counted as black particles in the aerial stem debris, was significantly highest when potato cvs Element, Mirka or Astarte were grown in the preceding two years (Table 3). In the debris after potato cv. Ostara the MS concentration was higher than after onion. No differences were found among other preceding crops and the fallow control. After initial infestation with PI the MS concentration in the debris was significantly higher than after infestation with FI. There was a positive correlation between ID and MS concentration in the debris. Per plot, the relative number of MS formed was highest after potato cvs Element, Ostara and Astarte. Potato isolate gave a higher relative number of MS than FI, whereas the ID had no significant effect.

Discussion

The ID in the fallow control plots shows that *V. dahliae* was able to survive during two years of fallow. For these plots, the increase of ID in time should be attributed to a gradual release of MS from the debris which carried the initial inoculum. Apparently the rate of release exceeded the rate of mortality of the MS. The lack of change in ID during the period D2-D3 indicates a balance between mortality of single MS and release of vital MS from plant debris. Probably, a decay has occurred in the next year caused by a faster rate of mortality than the rate of release of vital MS from the remnants of the debris. Therefore, the increase in ID found in cropped treatments as compared with the fallow control was caused by formation of 'new' MS of *V. dahliae* in plant debris.

Although the infestation levels established in the high level treatments were lower than the original 200 MS.g^{-1} soil, the effect of initial infestation level was still obvious. Crop yields on high level infested treatments were 7.5% lower than low level infested treatments in 1991, but no infestation level effects on yield were found in 1992, although differences in ID between the infestation levels were clear.

When the ID of the fallow control (being the ID released from the original inoculum) is subtracted from the ID measured in treatments cropped with potato, the differences in ID between low and high infestation levels are negligible. Although the growing season of 1991 was relatively short due to late planting, the observed tendencies of lower yields at the high infestation levels and the significant isolate effects indicate sufficient infection of crops. However, all plots seemed to be infected at the end of the season at both infestation levels. Probably, even the lower ID was above the threshold level that causes 100% infection in susceptible potato cultivars. The higher yields measured in soil with lower ID may have resulted from a later infection date (Nicot & Rouse, 1987). Earlier infection and colonisation were also indicated by a greener potato haulm on plots with a lower ID.

Isolate specificity in susceptibility to V. dahliae was observed for both field bean and potato. Isolate specificity was also observed for sensitivity in 1991 by the significant dry matter yield reductions of potato cv. Element and field bean when infected with their own isolates. Except for potato cv. Element, isolate specificity was only observed at the high infestation level. Probably for the other crops the low infestation level did not result in systemic infection. The differences in crop yields between isolates confirm the results of Krikun and Bernier (1987). Their isolates obtained from pea proved to be more pathogenic on pea than on potato. Tjamos (1981) found that isolates of *V. dahliae* from fields where tomato had been cropped were more pathogenic on tomato than isolates from fields where tomato had never been cropped, regardless

of the plant species used to obtain the inoculum. The current isolates were obtained from long-term monocultures and can be regarded as crop-specific isolates. However, after two years cropping with various crop species and cultivars, the isolate specificity towards potato cv. Element disappeared, whereas the effect on haulm yield in the fallow control treatment indicates that isolate specificity was intact. So, the crop species and cultivars did alter the MS population in the soil. This was also clearly shown for potato cv. Ostara, which had a build-up of the inoculum density similar to 'Element', but did not affect the potato yield in 1993 more than other crops or the fallow control.

Pea, flax, barley, sugar beet and onion did not affect the ID at the low infestation level compared to the fallow control. Only field bean and potato cvs Element, Ostara, and Astarte proved to be susceptible to *V. dahliae*. Since the potato cultivars responded differently to the two isolates and infestation levels, there seemed to be differences in sensitivity. Similar differences were found by Davis et al. (1983), Davis and Everson (1986), and Davis et al. (1990) working with USA potato cultivars. Potato cvs Element, Ostara and Astarte proved to differ in susceptibility, but the susceptibility of Mirka did not differ from the fallow control. However, on D3, the ID of Mirka at the high infestation level was similar to the ID of Astarte, which suggests an ID effect on resistance. Whereas the four potato cultivars differed in resistance, the cultivation of three of them in 1991 and 1992 reduced the potato yield in 1993. Only 'Ostara' as a preceding crop did not affect the yield more than the other crop species, while the ID in treatments previously cropped with 'Ostara' were as high as the ID in treatments cropped with 'Element'. So, virulence of MS formed also seemed to differ among potato cultivars.

Our results contradict the suggestion of Davis et al. (1994b) that resistance of the previously cropped potato cultivar can control the pathogen in a susceptible cultivar in a succeeding year. The high ID in their and our experiments after all cultivars grown indicate that resistance in a previous potato cultivar will not control the pathogen in a next sensitive crop. When the crop grown after the resistant potato cultivar, is moderately tolerant, cropping of a resistant potato cultivar could be a control measure.

Effects of both the ID and the isolate were shown for the MS formation in the debris of potato cv. Element grown in the third year. In potato, Davis et al. (1983) found similar results with a positive correlation between ID and aerial stem colonisation, and a negative correlation between ID and tuber yield. 'Ostara' has shown equal susceptibility for both isolates, but formed MS that seemed to be less pathogenic towards 'Element' than the cvs Element, Mirka and Astarte did. Counting by image analysis could lead to an overestimation, because of the

presence of particles other than MS of *V. dahliae* (Mol & Meijer, 1995), but relative differences are considered representative.

The effect of removal of the flax debris was significant in treatments with high PI infestation. Based on one-year experiments, Fitt et al. (1992) concluded that both fibre flax (i.e. pulled flax) and linseed (i.e. non-pulled flax) are susceptible for *V. dahliae*. In potato, Easton et al. (1972) found that burning of haulm could only decrease the infestation level of heavily infested soil by repeating the treatment for at least three years. These results agree with the current findings, and make removal of flax debris only valuable for integrated control of *V. dahliae* on a long-term. Since the removal of the flax debris was only significant on PI plots, also effectivity depends on the cropping history.

Decrease of the ID is sometimes attributed to exhaustion of MS by stimulation of the MS to germinate by exudates of plant roots, as suggested by Schreiber and Green (1963), but this does not agree with the results of this experiment, and recent work of Mol (1995b) who used the same crop species and cultivars. The finding that no crop species or cultivar was able to decrease the soil inoculum density, indicates that *V. dahliae* cannot be controlled in short crop rotations by use of any of the current crop species or cultivars as a 'luring' crop. This agrees with the statement that crop rotation is only a part of an integrated management system for controlling *V. dahliae* (El-Zik, 1985).

According to the results of the current experiment both the ID and the cropping history, including cultivar specificity, are key-factors in the effect of *V. dahliae* on susceptible and sensitive crop species and cultivars. However, none of them can control the ID to such an extent that yield damage is prevented. More research is needed on treatments of crop debris to control the formation of MS on crop debris and on removal of crop debris. Mol and Scholte (1995b; Mol et al., 1995b) have already experimentally proven that this concept is valid.

CHAPTER 6

EFFECTS OF CROP ROTATION AND REMOVAL OF CROP DEBRIS ON THE SOIL POPULATION OF TWO ISOLATES OF *VERTICILLIUM DAHLIAE*

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EFFECTS OF CROP ROTATION AND REMOVAL OF CROP DEBRIS ON THE SOIL POPULATION OF TWO ISOLATES OF *VERTICILLIUM DAHLIAE*

L. Mol, K. Scholte and J. Vos

Summary

Microsclerotia of *Verticillium dahliae* are produced in large numbers on senescing parts of host plants and remain viable in the soil for many years. Changes in the density of microsclerotia in the soil were measured in a micro-plot experiment with two isolates of *V. dahliae*, specific to either field bean or potato, several crop sequences comprising potato, field beans and barley, and either the removal of aerial debris of the crops or incorporation into soil.

Potato was more susceptible to the potato isolate and field bean more susceptible to the field bean isolate. Removal of debris of potato and field bean reduced numbers of microsclerotia in the soil in the subsequent years, but removal of barley straw had no effect. Initially non-infested control micro-plots became infected, probably by the growth of potato roots into the naturally infested subsoil. The rate of increase of the microsclerotia density in the non-infested control micro-plots was larger than in the initially infested treatments, because more colonised debris was produced. It is important to remove aerial debris of host crops in order to reduce the soil population of *V. dahliae*.

Introduction

Verticillium dahliae Kleb. is a main cause for reduced potato yields in short rotations (Scholte, 1990; Scholte & s'Jacob, 1990). The pathogen survives for several years in the soil by microsclerotia (MS). Microsclerotia are produced in senescing plant material of several crop species but especially in potato (Mol, 1995a). MS formation mainly occurs in the aerial parts of potato (Mol & Scholte, 1995a) and therefore, removal of the aerial crop debris from the field is potentially an effective measure to prevent the accumulation of MS in the soil (Easton et al., 1975; Takeuchi, 1987).

In this paper we report a study in which we compared the effect of removal of crop debris with the effect of incorporation into the soil on the MS population in the soil. The effect of the MS population on a crop varies depending on the provenance of the pathogen (Tjamos, 1981; Zilberstein et al., 1983b) whilst the quantity and the virulence of newly formed microsclerotia can be affected by the crop on which they were formed (Mol et al., 1995a) suggesting a degree of host-specificity in the pathogen. Therefore, two provenances of the inoculum were included in the experiment, namely inoculum isolated from fields with either continuous cultivation of potato or continuous cultivation of field bean. Changes in MS populations were recorded for a period of three years for different crop sequences including a susceptible potato cultivar, field bean and barley to illustrate the principle of haulm removal for good hosts and a poor host.

Materials and methods

Preparation of inoculum

Soil samples were taken from fields where either potato or field bean had been grown continuously for 14 years (P-soil) and for ten years (F-soil) respectively. Potato (*Solanum tuberosum* L., cv. Element) was planted in P-soil and field bean (*Vicia faba* L., cv. Alfred) in F-soil. From these plants *V. dahliae* isolates were obtained by plating stem segments on semi-selective pectate agar (Huisman & Ashworth, 1974) and pure cultures of *V. dahliae* established on potato dextrose agar. Two weeks later, the cultures were shaken with water and the suspension was used to inoculate pieces of green potato (potato isolate) or field bean (field bean isolate) stems previously sterilised by autoclave at 120°C for 20 minutes in Erlenmeyer flasks.

After two weeks of incubation at 23°C in the dark, large numbers of microsclerotia (MS) were present on the stem pieces. The stems of each plant species were air-dried and ground through a 1 mm sieve. Microsclerotia in subsamples of the ground material were quantified using a stereo dissecting microscope. The numbers of MS $gram^{-1}$ dry material were $9.5x10^5$ for potato isolate (PI) and $8.5x10^5$ for the field bean isolate (FI).

Micro-plots

A long-term factorial experiment with 144 micro-plots was laid out as a randomised complete block design with six replicates. Plastic containers of 75 l (60x40x32 cm) and holes in the bottom to allow drainage were buried in the centre of a 1.5x1.5 m plot on sandy soil in April 1991. A twice pasteurised mixture of sand and potting compost (2:1 by volume) (pH= 5.8, 10.3% organic matter) was used. Half the number of the containers was filled with soil

artificially infested with PI or FI inoculum in a density of 200 MS.gram^{-1} air-dry soil, and the other containers were filled with soil mixed with killed PI or FI inoculum.

In 1991, PI infested plots were grown with potato cv. Element (a sensitive cultivar: Scholte & s'Jacob, 1990) and FI infested plots were grown with field bean cv. Victor (Table 1). In 1992, potato, barley (*Hordeum vulgare* cv. Prisma) or field bean were grown after potato, and only potato was grown after field bean. In 1993, all plots were cropped to potato cv. Element.

The treatments consisted of removal or incorporation of aerial debris in 1991 and 1992. In the potato-barley and potato-field bean sequences, all four possible combinations of removal and incorporation into the soil of the debris of the crops were included. All combinations of infestation levels, isolates, crops and removal of debris resulted in 24 treatments.

Aerial plant debris was harvested and weighed and the dry matter content of the material was determined on a sample. If required, the remaining part of the plant debris was returned to the plots. In October, soil and plant debris were mixed using a concrete mixer, and each container was refilled with its own soil. All plant parts except the roots were removed in October 1993 to minimize the contribution of the potato crop grown in 1993 to the MS populations of *V. dahliae* in the soil. This was necessary to accurately assess the after-effects of the treatments in the previous years.

Plant densities were 4, 60, and 8 plants.plot^{-1} for potato, barley, and field bean, respectively. Plants were also grown in the area of 1.5x1.5 m directly surrounding the containers to create a crop situation.

Soil in the containers was analysed on N, P, K, and Mg concentration every year and the results were used to apply fertilizers at the required level. Weeds were regularly removed by hand from all plots and the whole field was irrigated when needed to prevent the plants from suffering of any water shortage.

Soil sampling and analysis

All plots were sampled with a 2.0 cm diameter auger to a depth of 20 cm in March 1992 (D1), October 1992 (D2), March 1993 (D3), October 1993 (D4), and March 1994 (D5) to measure the MS population of *V. dahliae* (Table 1). In October, the samples were taken before the aerial plant debris of the last crop was mixed through the soil. Each sample consisted of six cores and had a total weight of approximately 250 g air-dry soil. The samples were air-dried in the laboratory for two weeks and sieved through a 2.0 mm mesh.

For plating soil, a modified ethanol agar medium of Nadakavukaren and Horner (1959) was used. Just before pouring, per liter agar 5 ml ethanol 96% (vol/vol) and 50 mg chloro-

oxytetracycline was added to water agar (20 $g.l^{-1}$) that had been autoclaved for 20 min at 120°C and cooled to 50°C.

A 20 g subsample was taken from each soil sample and added to 90 ml 0.1% agar solution to make a 100 ml suspension (the viscous 0.1% agar solution was used to ensure a better distribution in the sample during mixing) and from each soil suspension 0.5 ml was spread over each of five Petridishes. These were incubated at high humidity (90-95% r.h.) and at a temperature of 22°C in the dark. After three weeks the soil was washed from the medium by gently rubbing and numbers of colonies of *V. dahliae* were counted using a stereo dissecting microscope (magnification 12x).

Results

During the whole experiment the infested treatments showed a higher MS population than the non-infested control plots (Table 1). At all sampling dates (D1-D5), removal of the potato debris clearly reduced MS population in the potato-potato sequence. Except at D5 in the initially infested treatments, removal of the potato debris in the potato-barley sequence also reduced MS population. Removal of the barley debris did not affect MS populations at all.

In the potato-field bean sequence a lower MS population was found after removal of the potato debris in 1991 at D1 and D5 in the non-infested treatments and at D1 to D4 in the infested treatments. At D5 lowest MS population was measured when both potato and field bean debris were removed. At this date, highest MS populations occurred when debris was not removed and removing either potato or field bean debris resulted in intermediate and equal levels.

In the non-infested FI treatments, no *V. dahliae* soil infestation was found at D1, but at later sampling dates *V. dahliae* was detected in the soil, and removal of crop debris tended to result in a lower soil infestation. In the infested treatments lower MS populations were observed when field bean and potato debris were removed, except at D2.

Except for the infested potato-field bean sequence, a peak in MS populations was observed 1 to 1.5 years after incorporation of the debris of potato or field bean into the soil (D2 and D5).

In 1991, a much larger amount of aerial debris was incorporated into the non-infested than in infested plots for both potato and field bean (Table 2). In 1992, a smaller amount was returned to the infested treatment in the potato-potato sequence, whereas in the other sequences no differences between infestation levels were found. In both years the amount of field bean debris returned to the field was larger than the amount returned of other crops.

Table 1. Influence of crop (P= potato, B= barley, F= field bean) and removal of aerial debris (r) on the number of colony forming units (cfu) of *Verticillium dahliae* per gram soil in March 1992 (D1), October 1992 (D2), March 1993 (D3), October 1993 (D4), and March 1994 (D5) without initial infestation or with infestation of the soil with microsclerotia.

Crop			Microsclerotial population (cfu.g^{-1})[c]									
1991	1992	1993	Not infested					Infested				
			D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
P[a]	P	Pr	7 a	48 a	20 a	36 a	107 a	37 a	108 a	47 a	72 a	126 a
Pr[a]	Pr	Pr	2 b	13 b	6 b	11 b	16 b	15 b	38 b	17 b	28 b	51 b
P[a]	B	Pr	7 a	45 a	24 a	18 a	34 a	37 a	129 a	48 a	47 a	33 a
P[a]	Br	Pr	7 a	45 a	30 a	18 a	34 a	37 a	129 a	53 a	38 a	50 a
Pr[a]	B	Pr	2 b	24 b	4 b	6 b	10 b	15 b	57 b	32 b	18 b	40 a
Pr[a]	Br	Pr	2 b	24 b	7 b	10 b	11 b	15 b	57 b	20 b	24 b	40 a
P[a]	F	Pr	7 a	24 a	7 a	11 a	18 a	37 a	56 a	36 a	38 a	70 a
P[a]	Fr	Pr	7 a	24 a	8 a	12 a	24 a	37 a	56 a	25 a	36 a	47 b
Pr[a]	F	Pr	2 b	27 a	3 a	5 a	11 b	15 b	28 b	11 b	16 b	45 b
Pr[a]	Fr	Pr	2 b	27 a	6 a	10 a	8 b	15 b	28 b	13 b	11 b	26 c
F[b]	P	Pr	0 a	8 a	5 a	19 a	39 a	66 a	34 a	73 a	42 a	199 a
Fr[b]	Pr	Pr	0 a	5 b	1 a	7 a[d]	16 a[d]	13 b	32 a	17 b	16 a[d]	28 b

[a]Infested plots infested with a potato isolate.

[b]Infested plots infested with a field bean isolate.

[c]Different letters indicate significant differences ($P<0.05$) between the treatments within a combination of crops in 1991 and 1992.

[d]Significant difference at $P<0.10$.

Discussion

In contrast to the potato plants, the field bean plants showed no symptoms of *V. dahliae* in the non-infested plots in the first year. Infection of the potato plants probably occurred in roots that penetrated through holes in the bottom of the container into the subsoil. In the past the field had been regularly cropped to potato, but field beans or related species had not been grown before. The MS population naturally present in the subsoil was therefore probably specific to potato. Specificity of a potato and a field bean isolate was also very evident in another experiment in the same field (Mol et al., 1995a). Tjamos (1981) and Zilberstein et al. (1983b)

Table 2. Amounts of aerial plant debris incorporated into the containers.

Crop		Amount of dry material (g.plot^{-1})				
1991	1992	Debris 1991 Infested			Debris 1992 Infested	
		No	Yes		No	Yes
Potato (haulm)[a]		107	39	***		
Potato (haulm)[a]	Potato (haulm)				50	29 ***
Potato (haulm)[a]	Barley (straw)				55	45 ns
Potato (haulm)[a]	Field bean (straw)				123	134 ns
Field bean (straw)[b]	Potato (haulm)	225	166	*	60	49 ns

*, ** and *** "Infested" significantly differs from "not infested" at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. ns = not significant.

[a]Infested plots infested with a potato isolate.

[b]Infested plots infested with a field bean isolate.

also found that the pathogenicity of *V. dahliae* was related to the cropping history. Specificity to potato of the suspected MS population in the subsoil also explains the delay in increase in MS populations in non-infested treatments with field beans as a first crop as compared to non-infested treatments with potato as a first crop (Table 1).

Upon infection, microsclerotial populations increased faster in the initially non-infested treatments than in the infested treatments. This can be explained by the larger amount of MS containing debris produced in the previously non-infested treatments as compared to deliberately infested treatments (Table 2). Scholte (1989) found that early in the growing season the percentage of potato stems infected by *V. dahliae* increased with increasing level of MS population, but the initial differences diminished later in the season. Davis et al. (1983) found a good correlation between MS population in the soil and the number of MS in stem tissue. With an earlier infection or with more infections, more MS can be produced, but the increase in number of MS will be smaller with each extra infection until a maximum is reached at 100% colonisation. Probably, maximal colonisation occurred in the infested treatments and then the amount of colonised debris determines the total number of MS per plant. In the current experiment, the amount of the debris was reduced by *V. dahliae* leading to fewer MS per plant in the infested treatments than in initially non-infested treatments. In the latter treatments a relatively high haulm production was probably attained because the colonisation, although probably complete at the end of the season, started later or progressed slower during the growing season.

Peaks in MS populations were measured 1 to 1.5 years after incorporation of the debris of a susceptible crop into the soil. So, the effects of MS from the colonised potato debris lasted at least one year. This agrees with the results of Ashworth et al. (1974) and Mol et al. (1995a). The latter authors observed an increase of the MS population over more than one year after mixing the soil with colonised ground potato stems. Also Huisman and Ashworth (1976) and Joaquim et al. (1988) found a sharp increase in MS population in the second year after growth of a susceptible crop, regardless whether the next crop was a non-susceptible or a susceptible host. The apparent increase in the year after incorporation may be explained by the disaggregation of plant debris containing MS.

For both infested and non-infested treatments, removing potato debris led to a lower MS population in the following years. Our results confirm those of Takeuchi (1987) obtained with Chinese cabbage and agree with Mol and Scholte (1995a) who found that the major part of the formation of MS occurs on aerial parts of potato cv. Element. Removal of barley straw did not affect the MS population, although production of MS can take place on both the aerial and the subterranean parts of barley (Mol, 1995a). Presumably, the release of MS from barley straw is so low compared to the release of MS from the potato debris of the previous year that removal of the straw did not have a measurable effect on the MS population. The lower MS population after removal of field bean straw agrees with results of Hoekstra (1989), who found that many MS can be produced on field bean straw. In the infested FI treatments, removing the debris of field bean resulted in a lower MS population in the next spring. At later dates both field bean and potato will have contributed to the increase of the MS population in this treatment.

The removal of plant debris did not prevent the increase in MS populations when only host crops were grown in succession. Therefore, removing potato haulm debris is unlikely to be effective as a method to control *V. dahliae* in short rotations of potato cropping, but it may be useful in rotations with a lower incidence of host crops.

CHAPTER 7

EFFECT OF HAULM TREATMENTS ON THE FORMATION OF MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE* ON POTATO

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L. Mol and K. Scholte

Summary

In four pot experiments, potato plants of cv. Element were artificially infected with *V. dahliae*. At an early and a late harvest haulms were killed chemically, by burning or by various other treatments, including cutting them into pieces of different lengths and keeping the debris on the soil surface or covering with soil. After 4 weeks the plant material was air-dried and the number of microsclerotia per mg was determined.

At the early harvest, in two experiments, the chemical treatment yielded more microsclerotia than the cutting treatments. Covering colonised haulm tissue with non-sterilised soil was effective in inhibiting microsclerotia formation. Shorter haulm pieces led to fewer microsclerotia at the later harvest if the material was kept on the soil surface. The variation in microsclerotial yield and in treatment effects among the different experiments was large.

Introduction

Formation of microsclerotia (MS) on potato plant debris is the most important survival mechanism of *Verticillium dahliae* Kleb. When infected plants senesce, the fungus leaves the xylem, readily permeates the surrounding tissues, and MS are produced in large numbers (Powelson, 1970).

Slattery (1981), Davis et al. (1983) and Mol and Scholte (1995a) reported large differences among potato cultivars in the number of MS.g^{-1} potato stem. Mol and Scholte (1995a) found that there was a 3-6 times greater production of MS when plants were harvested mature compared to harvesting 6 weeks before maturity.

For a proper assessment of the effect of aerial haulm treatments on the soil inoculum density and the control of the pathogen, the proportion of the reproduction on the separate organs of the

plant is important. In experiments with cvs Element and Mirka, Mol and Scholte (1995a) found that most MS production takes place on the aerial parts. Therefore it is relevant to examine possible ways to reduce the MS production on potato haulm. In this paper the results of four experiments are described in which the effect of different haulm treatments on the formation of MS on potato plant debris is studied.

Materials and methods

An isolate of *V. dahliae* was obtained from microsclerotia on stems of potato cv. Bintje from a commercial field of the Department of Agronomy in the autumn of 1990. Plants of cv. Element, which is susceptible and sensitive to *V. dahliae* (Scholte & s'Jacob, 1990), were infected with this isolate by immersing rooted sprouts in a suspension of blended pure cultures grown on potato dextrose agar for 2 weeks. Two sprouts were planted in a 10 l pot filled with quartz sand on 28 May 1991 and grown in a greenhouse under natural daylength and a temperature of 22°C (day) and 15°C (night) with a thermophase of 14 h.

The following quantities of nutrients were applied per pot, apportioned over 10 applications from planting date to 10 weeks after planting: 6.2 g N, 1.6 g P, 9.0 g K, 0.7 g Mg, and 40 ml of a trace element solution containing 20 g $MnSO_4.1H_2O$, 30 g H_3BO_3, 5 g $ZnSO_4.7H_2O$, 1 g $CuSO_4.5H_2O$ and 1 g $Na_2MoO_4.2H_2O$ per litre of water.

The first harvest (D1) took place 72 days after planting (DAP), at which time the first wilt symptoms were visible and the lower leaves had started to die. The second harvest (D2) was 105 DAP when the leaves were senescing but the stems were still green. On both dates three main treatments were applied to the aerial parts: a) plants were killed with the herbicide diquat, b) leaf blades of the plants were heated with a propane burner until the leaves wilted (burning) and c) aerial parts of the plants were harvested and treated in four different ways. These treatments consisted of all four possible combinations of two cutting methods and two post-cutting treatments. The haulm was cut into pieces of 5 or 20 cm. Cut pieces were kept in a permeable nylon bag either on the soil surface or covered with non-sterilized moist sandy soil (pH= 5.5; 2.7% organic matter). Before applying these treatments, the total weight of the haulm in each pot was assessed and the dry matter concentration of a sample was determined. The samples placed on the soil surface or covered with soil were kept moist by wetting the soil once a day. After 4 weeks the samples were air-dried and the loss of dry matter by decomposition was calculated. The plants of which the foliage was killed by diquat or by burning were left in their pots for 4 weeks to allow the stems to die. They were also wetted

once a day. All samples were ground, and the numbers of MS were determined. The experiment was laid out as a randomised complete block design with six replications (Exp. 1).

The experiment was repeated in 1992, 1993, and 1994 (Expts 2, 3 and 4), the sprouts being planted in potting compost (enriched peat soil) on May 13, April 13, and January 21 respectively. The amount of nutrients applied was lower than in Exp. 1 and amounted to 40, 30 and 30% of the amount in Exp. 1 for Expts 2, 3 and 4 respectively. In all these experiments an additional cutting method (pieces of 1 cm) was included. These experiments consisted of eight replications.

The first harvest dates (D1) were 50, 58 and 53 DAP and the second dates (D2) were 83, 98 and 91 DAP for Expts 2, 3, and 4, respectively.

In all experiments the MS were counted using an image analysis system as described by Mol and Meijer (1995). They estimated the MS based on the number of black particles after boiling small samples of ground plant material in a 1M sodium hydroxide solution. The MS densities in the debris were corrected for the yield loss by decomposition of the debris after harvest in order to compare the different treatments on a similar basis.

Results

On D1 in Expts 2 and 3, the chemical haulm treatment yielded significantly more MS per mg plant material than the cutting treatment in which 20 cm haulm pieces were kept on the soil suface (Table 1). The burning treatment showed inconsistent effects.

On D2 of Exp. 2, the haulm treated with diquat produced more MS per mg than the other treatments. In Exp. 4 the cutting treatments had more MS per mg than the chemical and the burning treatments, but numbers were very low compared to Expts 1, 2, and 3. Except for the burning treatment in Exp. 4, the yield of MS per mg was much higher on D2 than on D1. There were large differences between the experiments.

Except for D2 of Exp. 3 (dry matter concentration = 41.5%), plants were harvested when their haulm still contained a lot of water (Table 1).

Covering the haulm samples with soil after the harvest on D1 reduced the number of MS per mg by about 50% in Expts 1, 2, and 3, but had no effect in Exp. 4, where numbers were very low anyway (Table 2). Covering the debris with soil on D2 had no effect in Expts 1 and 2, but in Exp. 3 it reduced the number of MS by 20-70%. Covering the haulm with soil did not result in higher numbers of MS compared with keeping the debris uncovered.

Table 1. Number of microsclerotia per mg air-dried potato haulm after a chemical or a burning treatment or after leaving the debris on the soil surface in pieces of 20 cm. Data from two harvest dates (D1, D2) and four experiments; concentration of dry matter shown at the time of harvest.

Experiment	Microsclerotia (mg^{-1})						Dry matter (%)	
	D1			D2			D1	D2
	Chemical	Burning	Mechanical	Chemical	Burning	Mechanical		
1	24	24	12	43	49	63	12.0	16.5
2[a]	61 a	20 b	22 b	237 a	115 b	118 b	8.0	12.2
3[a]	158 a	124 a	21 b	331	237	304	8.5	41.5
4[a]	2 b	6 a	2 b	7 b	4 b	22 a	6.4	10.0

[a]Different letters indicate significant differences ($P<0.05$) between treatments of one experiment on one harvest date.

Table 2. Number of microsclerotia per mg air-dried potato haulm after leaving the debris on the soil surface or covering it with soil during 4 weeks after harvest. Data from two harvest dates (D1, D2) and four experiments. ns = not significant.

Experiment	D1			D2		
	Covered with soil		P≤	Covered with soil		P≤
	No	Yes		No	Yes	
1	20	12	0.05	48	32	ns
2	22	11	0.01	70	71	ns
3	22	6	0.001	333	262	0.05
4	2	3	ns	13	5	0.001

Table 3. Number of microsclerotia per mg air-dried potato haulm after leaving the debris in pieces of 1, 5, or 20 cm on two harvest dates (D1, D2). Data from four experiments. ns = not significant.

Experiment	D1				D2			
	Cutting length (cm)			P≤	Cutting length (cm)			P≤
	1	5	20		1	5	20	
1	-	21	11	ns	-	31	63	0.05[a]
2	16	15	17	ns	37	53	118	0.05
3	13	15	14	ns	306	389	304	ns
4	3	3	2	ns	5	12	22	0.05

[a]After log transformation.

There was no significant interaction between the effect of covering the haulm with soil and the length of the haulm pieces. Their length did not affect MS formation when covered with soil. When debris was left uncovered on the soil surface, there was also no effect on D1, but in Expts 2 and 4, there was an effect of the haulm length on D2 (Table 3). Haulm pieces of 20 cm yielded respectively thrice and twice as many MS per mg than the treatments with pieces of 1 and 5 cm, whereas in Exp. 1 this difference was not significant. In Exp. 3 no such effect occurred.

Discussion

Variation among the experiments was large. At the mature harvest date (D2) in Exp. 3 many more MS per mg plant material were produced than in the other experiments. This can be attributed to the more advanced maturity of the crop on the date of harvest, as indicated by the high dry matter concentration. In Exp. 4, however, MS numbers were very low. The dry matter concentrations of the haulm on the two harvest dates were also very low, probably because light intensity was low during crop growth.

In contrast to chemical haulm killing or burning, a cutting treatment leads to a sudden interruption in the water supply to the foliage. With the non-cutting treatments the time for the haulm to lose water will be longer. Fast desiccation of the haulm will limit the formation of MS (Ioannou et al., 1977c; Erwin et al., 1978). This explains why in our experiments haulm killing by cutting mostly inhibited the formation of MS.

Cutting the tissue into small pieces can also lead to faster desiccation of the plant material when left on the soil surface. In our experiments a negative effect of haulm cutting on MS formation was found on D2, but not on D1. Possibly the higher water content of the haulm at the earlier harvest date, in combination with the daily moistening, made suitable conditions for the multiplication of *V. dahliae*.

An extra factor causing some variation in the burning treatment is the treatment itself. Heating too briefly leads to a very slow killing of the haulm, whereas heating for too long may increase the temperature in the stem to a level that is harmful for the fungus.

The difference between Exp. 1 and Expts 2 and 3 could be caused by differences in nutrient supply (see Materials and methods). Davis and Everson (1986) got an indication from field surveys that an application of nitrogen could inhibit colonisation by *V. dahliae*, and Davis et al. (1990) observed a significant decrease in the number of colony forming units per g haulm tissue with increasing P concentration in the soil.

V. dahliae competes weakly with other organisms for its food source. Ioannou et al. (1977a) and Brinkerhoff (1969) reported that the numbers of MS produced in aseptic soil were four times greater than when formed during incubation in the presence of soil microflora. When colonised plant debris is covered with soil, *V. dahliae* starts producing MS and has an initial advantage over other soil micro-organisms, but after a very short time it has to compete with them. This could partly explain the negative effects of covering the plant material with soil. Another explanation could be the lower O_2 and the higher CO_2 levels in soil compared to the levels in air. Both low O_2 and high CO_2 concentrations have been shown to inhibit the formation of MS (Ioannou et al., 1977a, c), but when normal concentrations were restored the formation of MS resumed (Ioannou et al., 1977b). Possibly the delay in MS formation by changes in gas composition gave the soil micro-organisms a chance to colonise the buried plant material. The hypothesis that the shorter the pieces, the more closely will the debris be in contact with the soil micro-organisms and the more intense the competition with those organisms could not be proved in our experiments because burying shorter haulm pieces had the same effect as longer ones. Presumably competition by soil micro-organisms takes place on the surface of the plant tissue. At a more mature harvest *V. dahliae* had already begun to grow to the outside of the tissue and the competing organisms arrived too late to be able to prevent most of its reproduction.

The early harvest date coincides with the maturity of seed potato haulms when they are killed in The Netherlands. So, for seed potatoes, mechanical haulm killing together with incorporating the haulm into the soil can be recommended. At a late harvest date, mechanical haulm treatment would be useful if the plant material desiccated in the field very quickly and stayed dry for some time, unless the haulm was completely mature at the time of treatment.

CHAPTER 8

THEORETICAL APPROACH TO THE DYNAMICS OF THE INOCULUM DENSITY OF *VERTICILLIUM DAHLIAE* IN THE SOIL: A SIMPLE MODEL AND ITS FIRST TEST

depends on the cropping history, since the primary source of the inoculum is infested host debris. Plant roots can be colonised if microsclerotia germinate in the vicinity of the root tip (Fitzell et al., 1980). Colonisation is followed by systemic infection of the vascular system of the plant, whereafter *V. dahliae* is dispersed within the host by conidia and mycelial growth. Systemic infection by *V. dahliae* affects plant growth and depresses plant yield. Yield reduction is mainly caused by closure of the stomata and early senescence of the canopy after blockage of the vascular system of the haulm (Bowden et al., 1990; Haverkort et al., 1990). Microsclerotia are formed abundantly in infested tissue upon death of the host plant. Since *V. dahliae* has a very broad host range, the influence of other crops beside potato on this pathogen must be considered for all crops in a rotation.

The theoretical base for understanding the dynamics of the soil inoculum density of *V. dahliae* is still limited. In contrast to the extensive literature on experimental data and theoretical interpretations for disease progress curves for diseases induced by air-borne pathogens, the literature is much sparser for diseases induced by soil-borne pathogens, particularly with respect to theoretical interpretations. The temporal aspects of inoculum production, dispersal, and survival of soil-borne pathogens are very different. Mainly because of inconsistent results obtained by correlating the soil inoculum density to incidence or severity of the disease, little progress has been made in quantitative modelling of *V. dahliae* (Termorshuizen & Mol, 1995). Important factors contributing to the inconsistency are the gradual release of microsclerotia from the plant debris and the changing mortality of the microsclerotia over time.

In the current study, an equation is developed that models the inoculum densities of *V. dahliae* over long time spans (years) based on inputs of initial inoculum densities and crops. The number of systemic infections of plant roots during crop growth is related to soil inoculum densities. In turn, formation of microsclerotia in debris and reduction of the amount of the debris of different crops are related to the number of systemic infections. Finally, a gradual release and mortality of microsclerotia in the soil are included to calculate subsequent inoculum densities in the soil. The formation of microsclerotia and the production of crop debris are related to a series of observations on soil inoculum densities, to correlate soil inoculum density to crop yield and subsequent inoculum densities. The equation will be built based on measurable parameters and biologically and ecologically meaningful principles.

Model

In the model four effects and processes involving *V. dahliae* are distinguished: (a) reduction of the dry matter yield of crop debris by the pathogen; the size of this effect is related to the soil inoculum density, (b) formation of microsclerotia in debris; the number of new microsclerotia is related to the original soil inoculum density, (c) release of microsclerotia from decomposing crop debris, (d) survival of microsclerotia. Since inoculum densities in the soil are assessed by plating diluted soil samples on a selective medium, the variable measured is the number of colony forming units. We assume that there is only one reproduction cycle per year. The resulting inoculum density (M) at a certain time (t) is the result of the four effects and processes in each of the crops grown in the preceding years (Eq. 1).

$$M_t = C(I) \cdot Y(I) \cdot R_t \cdot S_t \cdot a \qquad (1)$$

M_t = inoculum density in the soil (#(colony forming units).g^{-1} soil) at time t;
C = concentration of microsclerotia in plant debris (#(microsclerotia).g^{-1} dry matter) as a function of the inoculum density (I) during crop growth;
Y = yield of crop debris ($g.m^{-2}$) as a function of the inoculum density (I) during crop growth;
I = inoculum density during crop growth (#(colony forming units).g^{-1} soil)
R_t = fraction of the microsclerotia released from debris which was incorporated into the soil at t=0;
S_t = fraction of viable microsclerotia of the total number which was incorporated with debris at t=0;
t = time after incorporation of the debris into the soil (years);
a = conversion factor relating the numbers in the tissue to the soil volume and density (g^{-1}(soil).m^2).

All abbreviations are explained in Appendix A.

Infection and disease severity

Both plant yield reductions and microsclerotial formation in debris are estimated by calculating the disease severity. When we assume a random distribution of the microsclerotia in the soil, the fraction of infected plants is related to the initial inoculum density. However, the fraction of plants infected does not seem to be a suitable parameter to predict the severity of the disease (Ashworth et al., 1972; 1979). In potato, Nicot and Rouse (1987) found that both the proportion of stems colonised and the percentage of vascular bundles colonised increased with increasing inoculum density, whereas Huisman (1988a) found a linear relationship between the inoculum density and the colonisation of field-grown cotton plants. The fraction of infected plants does not distinguish between early and late infections, and thus is not a good measure for disease severity. A better parameter would be the number of systemic infections per plant assuming a

linear relation between soil inoculum density and infections and a random distribution of infections over plants. This parameter will better represent the area under the disease progress curve. We assume that the severity of the disease of a plant is linearly related to the mean number of infections per plant that, in its turn, is proportional to the inoculum density:

$$N = c_I \cdot I \qquad (2)$$

N = mean number of systemic infections (#(infections).plant^{-1});

c_I = constant expressing the number of infections per unit inoculum density (#(infections).plant^{-1}) / #(colony forming units).g^{-1} soil);

I = inoculum density (#(colony forming units).g^{-1} soil).

Crop yield and disease severity

Infection of plants with *V. dahliae* leads to the inhibition of the flow of water and nutrients in the plant, and slows the production and translocation of photosynthates (Friebertshauser & DeVay, 1982). Johnson (1988) showed that the proportion of individual potato stems infected by *V. dahliae*, and the timing of infection are important for interpreting yield losses. Yield reduction of crop debris should correlate with the level of infection and consequently with the mean number of infections. This relationship, derived from appropriate differential equations, is expressed by the equation:

$$Y = Y_m \cdot e^{(-c_Y \cdot N)} \qquad (3)$$

Y = yield of aerial crop debris (g (dry matter).m^{-2});

Y_m = maximum attainable yield of crop debris (g (dry matter).m^{-2});

N = mean number of systemic infections (#(infections).plant^{-1});

c_Y = constant relating dry matter yield to the number of infections (plant.#$^{-1}$(infections)).

On the basis of Eq. (2), N can be substituted to relate the yield of the debris to the inoculum density (I) :

$$Y = Y_m \cdot e^{(-c_Y \cdot c_I \cdot I)} \qquad (4)$$

Wheeler et al. (1992) fitted a similar saturation type curve to correlate the tuber yield of potato with pre-planting densities of microsclerotia of *V. dahliae*.

Reproduction in host tissue

Formation of microsclerotia primarily occurs in infested host debris. *V. dahliae* is a very weak saprophyte and reproduction in plant debris not already occupied at the time of plant death is negligible. In living plants, the pathogen is restricted to the vascular system. Thus inoculum production by *V. dahliae* is effectively restricted to host tissue near colonised vascular tissue and for a relatively short time period after host death. The level of tissue colonisation, estimated by the mean number of infections per plant, should provide the best estimate of the level of inoculum production. The concentration of microsclerotia in crop debris is limited by the substrate availability. The inoculum formation, derived from appropriate differential equations, can be expressed by:

$$C = C_m \cdot (1 - e^{(-c_C \cdot N)}) \qquad (5)$$

C = concentration of microsclerotia in the crop debris (#(microsclerotia).g^{-1} (dry matter));

C_m = maximum attainable microsclerotial concentration (#(microsclerotia).g^{-1} (dry matter));

c_C = constant relating the concentration of microsclerotia in the debris to infections per plant (plants.#$^{-1}$ (infections)).

Substitution of N results in:

$$C = C_m \cdot (1 - e^{(-c_C \cdot c_I \cdot I)}) \qquad (6)$$

Time, decomposition and survival

In this model we will use a thermotime, since temperature will be a major factor in the decomposition of debris and the survival of microsclerotia. Time will be expressed as degree-days with a minimum temperature of 0°C as used for processes describing decomposition of debris in the soil (Honeycut & Potaro, 1990). Degree days were derived from daily minimum and maximum air temperatures at Wageningen. For a single day the temperature sum was obtained by the mean of the daily minimum and the daily maximum temperatures, both with a minimum of 0°C. To express the time variable in units more compatible with the annual nature of cropping, the degree days were normalised to larger thermotime units by dividing the degree days by 3,600, the mean number of degree days per year for 1991-1993. Thus one thermotime unit represents 3,600 degree days and the time starts when the debris is incorporated into the soil.

Decomposition and inoculum density in the soil

Release and dispersal of microsclerotia from infected plant debris is a major process affecting soil inoculum densities (Huisman, 1988a). Since colonisation of roots by *V. dahliae* depends on random encounters between immobile microsclerotia and expanding root systems (Huisman, 1982; Huisman & Gerik 1989), the colony forming unit, either a single microsclerotium or an aggregate of microsclerotia, is the most relevant measure of inoculum density. Current soil assay methods also measure inoculum density in terms of colony forming units.

Decomposition will be gradual over time. Initially, after incorporation of the plant debris into the soil, the more readily degradable components will be decomposed and the remaining material will contain aggregates of microsclerotia. Subsequently the aggregates are further degraded to release single microsclerotia. Our assumption is that this complicated process can be approximated by a two step decomposition and dispersal process with different rates: (a) decomposition of easily decomposable components in fresh debris leading to aggregates of microsclerotia in the remnants, (b) decomposition of the remnants of the debris containing the aggregates of microsclerotia.

The fraction of the number of microsclerotia in aggregates (A) in relation to the total number of microsclerotia in debris at time t after incorporation into the soil can be described by:

$$A = 1 - e^{-c_A \cdot t} \qquad (7)$$

A = fraction of the number of microsclerotia in aggregates;

t = thermotime (3,600 degree days);

c_A = constant relating the fraction of microsclerotia in aggregates to thermotime (per 3,600 degree days).

With the decomposition of the less easily degradable organic material, the microsclerotia will be released in the soil as single microsclerotia. The number of released microsclerotia in relation to the total number of microsclerotia produced in plant tissue and added to the soil (R), can be described by the differential equation:

$$\frac{dR}{dt} = c_R \cdot A \cdot (1 - R) \qquad (8)$$

R = fraction of the number of microsclerotia released;

t = thermotime (3,600 degree days);

A = fraction of the number of microsclerotia decomposed to aggregates;

c_R = constant relating the fraction released microsclerotia to thermotime (per 3,600 degree days).

The fraction of microsclerotia in the aggregates and an increasing persistence of the remnants of the debris (1 - R) affect the rate. Substitution of A and integration leads to:

$$R = 1 - e^{-c_R\left(t + \frac{e^{(c_A \cdot t)}}{c_A} - \frac{1}{c_A}\right)} \qquad (9)$$

Survival and inoculum density in the soil

Commonly observed surviving fractions of microbial populations in relation to time are exponential declines. We surmise that a similar situation might apply for microsclerotia of *V. dahliae* and the fraction survival (S) of the microsclerotia as a function of the time (t) can then be described by:

$$S = e^{(-c_S \cdot t)} \qquad (10)$$

S = fraction of the microsclerotia that survived;

t = thermotime (3,600 degree days);

c_S = constant relating the survival of microsclerotia to time (per 3,600 degree days).

Effect of different crops and cultivars

The model needs to account for major differences in the susceptibility and sensitivity to systemic infection among plants. A first treatment factor (f_I) is included in Eq. (2) to allow for those differences:

$$N = f_{Ik} \cdot c_I \cdot I \qquad (11)$$

N = mean number of systemic infections (#(infections).plant^{-1});

f_{Ik} = crop specific factor related to susceptibility and sensitivity;

I = inoculum density (#(colony forming units).g^{-1} soil);

k = specific crop.

Because of differences in biomass production, a second crop factor (f_Y) should be included to adjust for the fact that the maximum attainable yield (Y_m) will not be the same for all crops. The combination of $f_Y.Y_m$ represents the maximum attainable yield for a crop species or cultivar. Thus Eq. (4) can be modified to include this second factor:

$$Y = Y_m \cdot f_{Yk} \cdot e^{(-c_Y \cdot N)} \qquad (12)$$

f_{Yk} = crop specific factor related to the maximum attainable yield;

k = specific crop.

Observed inoculum density

The number of colony forming units observed in the soil (M) can be described by the combination of equations (1), (4), (6), (9), (10), (11) and (12). For the calculation of M, the cropping history during a certain time (j) is taken into account. The formula to fit for M is then:

$$
\begin{aligned}
M &= \sum_{t=0}^{t=j} (C.\ Y.\ R.\ S.\ a) \\
&= \sum_{t=0}^{t=j} (C_m \cdot (1 - e^{(-c_C \cdot f_I \cdot c_I \cdot I)}) \\
&\quad \cdot f_Y \cdot Y_m \cdot (e^{(-c_Y \cdot f_I \cdot c_I \cdot I)}) \\
&\quad \cdot (1 - e^{-c_R (t + \frac{e^{(c_A \cdot t)}}{c_A} - \frac{1}{c_A})}) \\
&\quad \cdot e^{(-c_S \cdot t)} \cdot a)
\end{aligned}
\qquad (13)
$$

a = factor converting the number of microsclerotia in the debris to the soil volume (g^{-1}(soil).m^2).

Data sets

The equations were fitted to the datasets from a micro-plot field experiment in which a known amount of inoculum was introduced into the soil before the first cropping season (Mol et al., 1995a). Crop species and cultivars included in the experiment were potato cvs Element, Ostara, Mirka and Astarte; flax (*Linum usitatissimum* cv. Viking); Pea (*Pisum sativum* cv. Finale); spring barley (*Hordeum vulgare* cv. Prisma); sugar beet (*Beta vulgaris* cv. Univers); onion (*Allium cepa* cv. Jumbo); field bean (*Vicia faba* cv. Victor) and a fallow control which is treated in the model as a totally immune crop. Each crop was grown on the same plot for two subsequent years. In the third year, the highly susceptible potato cv. Element (Scholte & s'Jacob, 1990) was grown on all plots (including the fallow plots). Each year, dry matter yields of aerial crop debris were measured and the inoculum density in the soil was determined twice a year. The first sampling date was 0.16 thermotime unit (6 months) after incorporation of the debris of the first crop in late fall. The last sampling date was 0.16 thermotime unit after incorporation of the debris of the third crop. The experiment comprised five replications.

Calibration

Equation (13) and the subformula for yield (Eq. 4) were put in a computer programme which optimizes an equation by minimising the residual sum of squares. Differences between observed values for debris yield and soil inoculum densities of the individual plots and those predicted by the model equations with a given set of constants were the bases for the residual sum of squares. At programme initiation, all unknown constants were given an arbitrary value. The only other input values were the "known" inoculum densities at rates of 2 and 200 microsclerotia.g^{-1} soil, introduced into the soil at the start of the experiment. Values for constants were adjusted by optimizing the value of a single coefficient or treatment factor values sequentially. This process was repeated many times until the minimum of the sum of squares was reached. The possibility to fix the values of a subset of parameters during calculations using Eq. (4) or Eq. (13) was built into the programme. To estimate the inoculum density at a given time, the contributions of each of the previously grown crops were calculated separately and then summed for each field plot. The initial inoculum introduced into the soil used in the field trial consisted of ground infested potato stems. The major part of this inoculum was still in the form of (highly) aggregated microsclerotia. Consequently, the number of colony forming units to which the first crop was exposed was not known. To calculate the number of colony forming units during the first growing season, the release and survival functions (Eqs 9 and 10), utilizing the values for the constants derived by the process of minimising the residual sum of squares, were applied to the initial inoculum. The inoculum was arbitrarily set to be applied in the fall of 1990 (t_0) with no dispersal of microsclerotia in aggregates and 100% viability.

Equation (13) contains many unknown variables. Calibration of all parameters simultaneously could lead to inaccurate estimates. Therefore, we first fitted Eq. (4) describing effects on the yield of crop debris with observed debris yields of the field experiment. With those results we obtained the value for the maximum attainable yield and f_Y for the crops used. After doing so, we fitted Eq. (13). The value of f_I for the most susceptible crop was arbitrarily set at 1.0. This method will allow ready comparison among crops, since their f_I values will be expressed as a fraction of the f_I value of the most susceptible crop.

One model run consisted of several runs of Eqs (4) and (13) alternately until the estimated values of the parameters reached a constant value. During optimisation of Eq. (4) c_Y, Y_m and f_Y were estimated whereas these parameters were fixed during optimisation of Eq. (13). When Eq. (13) was optimised, c_I, c_C, C_m, and f_I were estimated. Because c_I and c_Y, and c_I and c_C cannot be estimated independently in the model, we will only present the products of $c_I.c_Y$ and $c_I.c_C$.

Results

The model was fitted with the computer programme to two data sets to arrive at values for the parameters in Eq. (13) which needed quantification. In the first calculation of optimal values for constants (Run 1), the observations from spring 1992 until fall 1993 representing four data points on inoculum densities and two data points for debris yield for each plot were used. In the second calculation of optimal values for constants (Run 2), the observations on inoculum densities were extended to the spring 1994 data and included the contribution of the debris of potato cv. Element grown in 1993 to the inoculum density.

The correlations between the observed and estimated values were very high for both the yields (R^2 = 0.72-0.80) and the inoculum densities (R^2 = 0.60-0.91; Fig. 1a) in the two runs (Table 1). In Runs 1 and 2 similar values were obtained for constants of the equations (C_m, $c_I.c_Y$, $c_I.c_C$, c_A, c_R, c_S, f_I, and $Y_m.f_Y$). The maximum concentration of microsclerotia in debris (C_m) was ca 130. The value observed for $c_I.c_C$ (ca 0.8) was much higher than that for $c_I.c_Y$ (ca 0.01), reflecting a much higher initial rate of inoculum formation in tissue than the rate of reduction in haulm production in relation to soil inoculum density.

Compared to potato cv. Element (f_I = 1.0) the factor values for infection (f_I) were much lower for most crop species and cultivars (Table 1). Potato cv. Ostara had a factor value of approximately 0.5; potato cvs Mirka and Astarte and flax had similar values of ca 0.04. Even lower were pea, barley, onion and sugar beet (ca 0.007), whereas field bean was by far the lowest (0.0002). In the inoculum production function, crops with high values for f_I (potato cv. Element) reached maximum inoculum formation at very low initial inoculum densities (5-10 microsclerotia.mg^{-1} soil) while those with low f_I values (potato cv. Mirka) had response curves with much lower initial gradients (Fig. 1b). Yield reduction functions, in contrast, did not show a strong response to soil inoculum density (Fig. 1c).

The residue decomposition and inoculum survival functions, when fitted with the values obtained for c_A, c_R and c_S (ca 0.6, 0.6 and 0.5) generated curves that indicated that at least four thermotime units were needed for dispersal of most of the inoculum produced in tissue (Fig. 1d). With those values only 13% of the microsclerotia will have been released after one thermotime unit and the maximum inoculum density will be reached ca. 2.3 thermotime units after incorporation of the debris into the soil (Fig. 1d). With a c_S of 0.51 it will take 1.4, 4.5, 9.0 and 13.5 thermotime units until 0.50, 0.90, 0.99 and 0.999 of the original input has died, respectively.

Table 1. Values for parameters, crop specific factor values and the relevant coefficients of correlation obtained after fitting field data of 10 crop species and cultivars from 2 years in Eqs (4) and (13). Abbreviations and units are explained in the text and in Appendix A.

Parameter	Run (1)[a]	Run (2)[b]	Factor related to
C_m	127	131	Maximal microsclerotial concentration
$c_I \cdot c_Y$	0.013	0.011	Yield reduction
$c_I \cdot c_C$	0.85	0.68	Microsclerotial formation
c_A	0.59	0.60	Aggregates
c_R	0.58	0.64	Release
c_S	0.51	0.59	Survival
Crop specific factors			
Crop	f_I Run (1)	f_I Run (2)	$Y_m \cdot f_Y$ [c]
Potato 'Element'	1.00	1.00	137
Potato 'Ostara'	0.53	0.56	128
Potato 'Mirka'	0.04	0.05	137
Potato 'Astarte'	0.03	0.05	162
Flax	0.04	0.04	232
Pea	0.01	0.02	192
Barley	0.008	0.01	234
Sugar beet	0.007	0.01	182
Onion	0.005	0.01	126
Field bean	0.0002	0.0002	229
Coefficients of correlation			
	Run (1) Eq. 13	Run (2) Eq. (13)	Eq. (4)
R^2 (individual plots)	0.66	0.60	0.72
R^2 (means of treatments)	0.91	0.90	0.80

[a]Based on inoculum densities of spring 1992, fall 1992, spring 1993 and fall 1993, and the yields of aerial debris of 1991 and 1992.

[b]Based on inoculum densities of spring 1992, fall 1992, spring 1993, fall 1993 and spring 1994, and the yields of aerial debris of 1991, 1992 and 1993.

[c]Values, representing maximum yield per micro-plot, are the means obtained from 1991 and 1992 (one micro-plot equals 0.12 m^2).

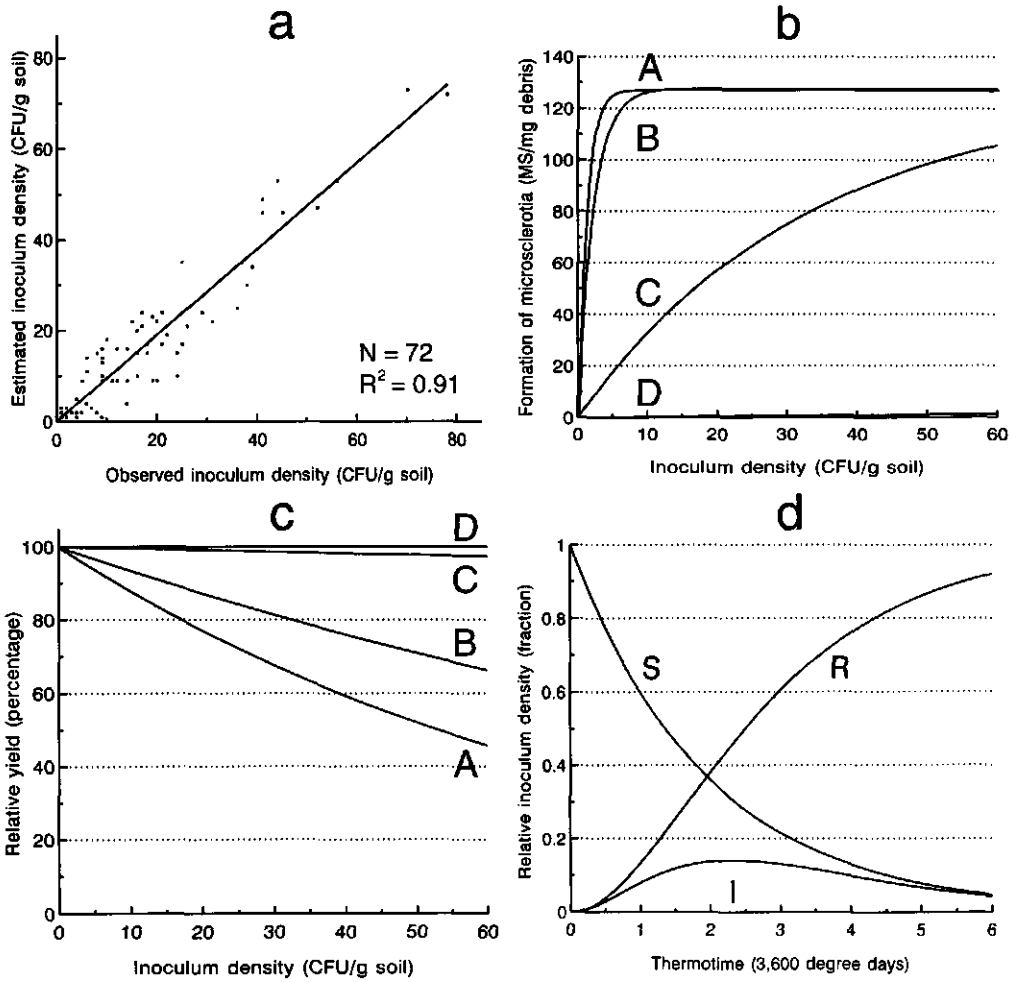

Fig. 1. Correlation between the observed and estimated number of colony forming units (CFU) per g soil for the means of five replications of Run (1) (a), curves for formation of microsclerotia in crop debris (b) and relative crop yields (c) as a function of the soil inoculum density as obtained with the parameters calculated for potato cvs Element (A), Ostara (B) and Mirka (C), and field bean (D), and the release (R) and survival (S) of microsclerotia from plant debris and number of CFU(I) over time (calculated with the following values for the constants c_A, c_R and c_S: c_A=0.59; c_R=0.58; c_S=0.51) (d).

Maximum attainable yields were estimated for the different crop species and cultivars ($Y_m.f_Y$; Table 1) showing the lowest values for potato and onion and highest values for flax, barley and field bean. Sugar beet and pea had intermediate values.

Calibration of the model was quite sensitive to small changes in inoculum density. When the contribution to soil inoculum from the third potato crop is ignored for the spring 1994 data points, the values derived for the constants are considerably different from those observed when it is included (Run 2). The crop specific factor f_I increased for most crops. The value for C_m was estimated to be 208 ms.mg^{-1}. While the third crop makes only a small contribution to soil inoculum (t = 0.16 thermotime unit), its inclusion resulted in values for the constants which closely matched Run (1).

Once values had been derived for the parameters, the model was used to predict the concentration of microsclerotia in the debris of the various crop species and cultivars in a soil with 2.3 colony forming units g^{-1} soil. The estimates for the concentrations of microsclerotia obtained compared well with those measured over a wide range of values by Mol (1995a) for plants grown under similar inoculum level in a separate pot experiment (Table 2). Mol (1995a) infested the soil with 30 microsclerotia per g soil in ground potato tissue. With the constants for release and survival the effective inoculum density will have been 2.3 microsclerotia g^{-1} soil.

Table 2. Estimated concentrations of microsclerotia on aerial crop debris at a soil inoculum density of 2.3 colony forming units per g soil and values observed by Mol (1995a).

Crop	Microsclerotial concentration (microsclerotia.mg^{-1})	
	Estimated	Mol (1995a)
Potato 'Element'	107.5	91.2
Potato 'Ostara'	81.6	83.3
Potato 'Mirka'	8.5	47.3
Potato 'Astarte'	7.4	55.7
Flax	8.8	7.6
Pea	2.4	3.3
Barley	1.9	2.2
Sugar beet	1.7	2.8
Onion	1.3	2.9
Field bean	0.1	2.8

Discussion

The results observed indicate that the model can adequately describe the population dynamics of *V. dahliae* in experimental field plots. The values for inoculum densities and yields, predicted by the model are calculated solely from the initial inoculum added to soils and values of constants derived from the calibration. The equations were based on ecological considerations and were not selected for a best fit to the experimental data. The good correlation between observed and estimated values indicate that the assumptions made are compatible with the data measured (Fig. 1a). A perfect correlation between estimated and observed values cannot be expected. The observed data certainly contain 'noise' due to a number of uncontrolled conditions or because our assumptions may be too simplistic for the complex processes involved in the dynamics of the inoculum density of *V. dahliae*.

Values derived for a number of constants were consistent with values observed in other trials. The maximum concentration of microsclerotia in the plant debris was estimated to be approximately 130. This agrees with the value of 160 microsclerotia.mg^{-1} tissue observed for artificially infested debris of potato cv. Element grown under similar environmental conditions (Mol, 1995a). With the derived constants, the model estimated concentrations of microsclerotia in tissues of the various crops (including wilt-immune species) which were in close agreement with actual values measured in tissue of plants grown in moderately infested soil. Only values predicted for potato cvs Mirka and Astarte and field bean differed markedly. The reasons for these discrepancies are not clear. The estimated mortality rate leads to a 40% reduction of the inoculum after one thermotime unit and 60% after two thermotime units (Fig. 1d). Such values are in line with attrition rates observed by other workers (Huisman & Ashworth, 1976; Ben-Yephet & Szmulewich, 1985). The shape of the release and survival functions (Fig. 1b) describe a much more dynamic population of microsclerotia in the soil than observations on soil inoculum density over a long time span would indicate. Because of a slow release rate and a long survival a near steady state of the observed inoculum density is reached over a period of years. At the maximum inoculum density, reached at 2.3 thermotime units, only 14% of the total production of microsclerotia was estimated to be detected by plating.

The model calculated a systemic infection level for all crops, including wilt-immune ones. Strict interpretation of infection levels could be misleading. In the model all formation of microsclerotia is attributed to the aerial parts after systemic infection by *V. dahliae*. However, a part of the formation of microsclerotia will take place in subterranean parts after systemic infection. In potato, Mol and Scholte (1995a) found that ca 25% of the reproduction took place at subterranean plant parts. In many crops microsclerotia can also be produced in root tissue

without systemic infection. The cortical root tissue of almost all plants are readily colonised by *V. dahliae* (Evans & Gleeson, 1973; Huisman, 1988b). If systemic infection is small, non-systemic formation of microsclerotia may represent a significant proportion of the total production (Mol, 1995a). Thus, when the model calculates a very low infection level, it may signify some inoculum production on root tissue rather than a true systemic infection level. The predicted systemic infection levels for the immune crops were low, generally less than one percent of the values observed for the highly susceptible potato cv. Element. Even with wilt-immune plants, there may be some movement of *V. dahliae* into a portion of the aerial vascular system (Mathre, 1989; Krikun & Bernier, 1990). Mol (1995a) found microsclerotia on the aerial debris of all crop species and cultivars after they had been grown on infested soil. The calculated microsclerotial concentrations, at an inoculum density of 2.3 colony forming units g^{-1} soil, are very similar compared to the concentrations he found (Table 2).

The crop specific factor f_l provides an estimate of the relative contribution of various cultivars to inoculum build-up in tissue and in soil. The factor should primarily correct for differences in susceptibility and sensitivity to systemic infection and for microsclerotia formation in debris. At low initial inoculum densities, the rates of inoculum build-up for potato cv. Element are about twice as fast as that for the moderately resistant 'Ostara' and ca 25 times as fast as for the highly resistant 'Mirka' and 'Astarte'. The differences would diminish at higher inoculum densities due to the nature of the function for the formation of microsclerotia (Fig. 1c). Davis et al. (1994b) have reported differences in systemic infection and formation of microsclerotia among potato cultivars. Among resistant and susceptible cotton cultivars there were differences in the number of propagules and the increase of the number of propagules over time when they were artificially infested in the tap root (Harrison & Beckman, 1982). The values for the f_l factor for Mirka and Astarte were only three to five times higher than those calculated for immune crops (Table 1) which is consistent with their status as highly resistant cultivars (Scholte & s'Jacob, 1990). The high specificity of the potato isolate of *V. dahliae* used in these studies (Mol et al., 1995a) is supported by the low f_l values for flax (0.04) and field bean (0.002). The value for flax is similar to that obtained for potato cv. Mirka, while field bean proved to be the most resistant with respect to inoculum build-up of a potato isolate.

Our model uses a single upper limit for microsclerotial formation in plant tissue. This assumes that plant debris of all crops is equally suitable as a substrate for formation of microsclerotia once colonised by *V. dahliae*. It is not known whether this is true or not, but inoculum has been grown on plant material from different sources with good results (Termorshuizen & Mol, 1995).

The yield reduction function may need further adjustments. The model currently assumes that yield losses of debris can approach 100% at high inoculum levels. This may not actually be the case. In potato,wilt symptoms or growth reductions of haulm are rarely observed prior to tuber initiation (Busch & Edgington, 1967). Since a major part of the canopy has been formed by that time, yield reduction due to Verticillium wilt could have an upper limit far below 100%. This feature may be responsible for the shape of the relative yield curves predicted by the model which appear flatter than is expected in an exponential function and probably underestimate losses at moderate inoculum densities. The maximum yields could well be estimated by a crop growth model and they could be linked to the calculations to obtain improved estimations.

Many of the constants used in our model compact the effect of a number of variables into a single factor. This is useful for simplification and acceptable as long as these variables are constant in the data set evaluated by the model. Some of the variables embedded in the $c_l.c_C.f_l$ constant are root colonisation rates (Evans & Gleeson, 1973; Huisman, 1988b), plant densities (Brody et al., 1990), root densities (Huisman, 1988b) and different treatments of aerial debris (Mol & Scholte, 1995b). The release and survival constants include a number of variables that will not be the same from field to field. For example, Ashworth et al. (1974) found that cultural practices and soil moisture affected the rate of release of microsclerotia. Under rototillage and moist conditions most of the microsclerotia were free after one year. In the dry or non-tillage conditions, up to 90% of the viable microsclerotia were still bound in infected cotton debris after one year.

The model does not allow for a differential effect of subsequent crop debris (directly or indirectly) on inoculum already in the soil. Our simplifying assumption was that soil microlife is very concentrated at the place where recently incorporated organic particles are situated and that the distance between particles and existing inoculum is large enough to make the interaction small. However, Davis et al. (1994a) found that subsequent crop residues can affect the infection rate by *V. dahliae* and also disease severity. Reductions in wilt were correlated with soil microbial activity (Davis et al., 1994a). Effects of differences in biological activity have been attributed to colonisation and the infection of the roots (Jordan et al., 1972). When the influence of the soil microbial activity is better defined, it could be included in the model at the infection level.

Extension of our model to other situations will show different values for the constants due to the above mentioned and other variables. More ideally, as we gain more quantitative information about the effect of such variables, the constants in the equation can be expanded to include separate values for these other components.

For use in a management system, a simple model, like the current one, could be sufficient if applicable to a broad range of cropping circumstances. For that purpose, the model should be tested under a variety of conditions.

Estimations of the development of the inoculum density as influenced by crop species and cultivars over a long time span could be a tool to get a better view on the expectations on effects of *V. dahliae* on subsequent crops. The model can be used to calculate the likely consequences of cropping sequences for the soil inoculum density. The assumptions, of course, are that field environmental conditions are similar to those in the test field plots. Large differences are calculated when potato cv. Element is grown continuously, once in two, three, six, and twelve years in rotation with a crop supporting very low microsclerotial formation, as for field bean in our plots (Fig. 2). Due to large reductions in yield of crop debris and a slow release and slow attrition, a peak will be reached within a short time, followed by a steady state

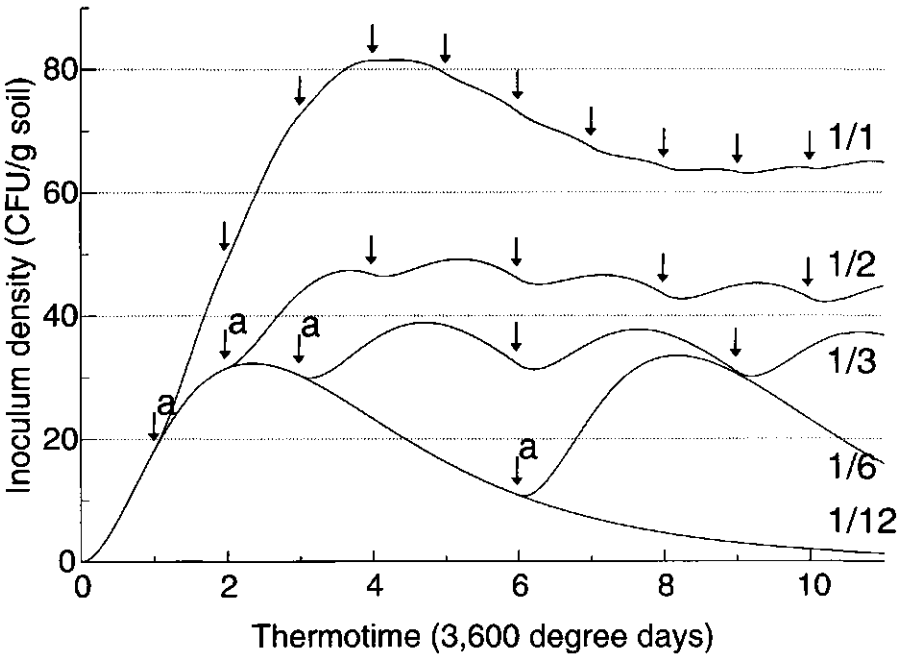

Fig. 2. The estimated inoculum densities after cropping potato cv. Element continuously (1/1), or once in two (1/2), three (1/3), six (1/6) or twelve (1/12) years in rotation with field bean using the parameters obtained in Run (1). The first crop debris is incorporated into the soil at t=0 after being grown in soil with an inoculum density of 5 colony forming units (CFU) per g soil. The time of incorporation of debris of potato cv. Element is indicated with an arrow (*arrow only applies to the up-going line).

at a high inoculum density. For a susceptible crop, the largest contribution to the soil inoculum density is reached during the second growth season after incorporation of its debris into soil. In the once in two years crop rotation the susceptible crop was grown in the season with the highest inoculum densities and the largest yield losses could be expected. Since the mortality and release rates of microsclerotia are low, cropping 'low-susceptible' crops should be done continuously over many years to control *V. dahliae*. In view of the calculated dynamics for soil inoculum densities, a highly susceptible crop should not be grown more frequently than once in eight years.

Knowledge of the influence of crops and cropping sequences on pathogen populations is important to develop sound production systems. Our model presents a first approach for making long term predictions for *V. dahliae*. It has been shown that inoculum densities can be described over a long time span by our model. The output matched the observations found by other workers. With the coefficients obtained, relative differences among crops can be quantitatively compared. The model also allows us to begin to examine the consequences of long term cropping sequences on the soil inoculum. The model needs to be tested (and calibrated) for a range of different circumstances before it can have a wider application.

Appendix A

Variables and parameters used in the equations

A = fraction of the number of microsclerotia in aggregates.
C = concentration of microsclerotia in the plant debris (#(microsclerotia).g^{-1} (dry matter)).
C_m = maximum attainable microsclerotial concentration (#(microsclerotia).g^{-1} (dry matter)).
I = inoculum density during crop growth (#(colony forming units).g^{-1} soil).
M = inoculum density in the soil (#(colony forming units).g^{-1} soil).
N = mean number of systemic infections (#(infections).$plant^{-1}$).
R = fraction of the number of microsclerotia released.
S = fraction of viable microsclerotia.
Y = yield of aerial crop debris (g (dry matter).m^{-2}).
Y_m = maximum attainable yield of crop debris (g (dry matter).m^{-2}).

a = conversion factor relating the numbers in the tissue to the soil volume (g^{-1}(soil).m^2).
c_A = constant relating the fraction of microsclerotia in aggregates to thermotime (per 3,600 degree days).
c_C = constant relating the concentration of microsclerotia in the debris to infections per plant (plants.$\#^{-1}$(infections)).
c_I = constant expressing the number of infections per unit inoculum density (#(infections).$plant^{-1}$)/(#(colony forming units).g^{-1} soil).
c_R = constant relating the fraction released microsclerotia to thermotime (per 3,600 degree days).
c_S = constant relating the survival of microsclerotia to thermotime (per 3,600 degree days).
c_Y = constant relating dry matter yield to the number of infections (plant.$\#^{-1}$(infections)).
f_I = crop specific factor related to susceptibility and sensitivity.
f_Y = crop specific factor related to the maximum attainable yield.
j = thermotime after incorporation of the debris into the soil (3,600 degree days).
k = specific crop.
t = thermotime after incorporation of the debris into the soil (3,600 degree days).

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

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Reduction of soil infestation by means of lure crops

Experimentation with root observation boxes yielded a quantitatively reproducible description of the influence of plant roots on the germination of microsclerotia of *V. dahliae*. All the crops studied seemed to stimulate germination of microsclerotia of *V. dahliae* to some extent, but hosts (potato, field bean) had a stronger stimulating effect per root tip than non-hosts (barley). This confirms the results of Schreiber and Green (1963) and Fitzell et al. (1980). The germination was highest at the root surface, but decreased with increasing distance between the root tip and microsclerotia. In barley the stimulating effect extended to 1 mm from the root surface, but roots of hosts still showed an effect at greater distances.

The luring effect of crops was estimated by including the root density of each crop in the calculations. These calculations showed that no crop species or crop cultivar was able to decrease the soil inoculum density to an extent that could be exploited in practice. Thus, *V. dahliae* cannot be controlled in short crop rotations by using any of the crops investigated as a 'lure' crop. The main reasons for the small effects of roots on the soil inoculum density are the low number of hyphae formed per microsclerotium in the sphere of influence of the roots (<0.5 hyphae per microsclerotium) and the low percentage of the volume of the soil that is affected by roots ($<37\%$).

Contribution of crops to soil infestation

Microsclerotia were found on the aerial debris of all crops investigated grown in infested soil. In various host crops (potato, flax), the density of microsclerotia in the plant material was highest in the aerial parts; this indicates systemic colonisation. Large differences were found in the formation of microsclerotia among potato cultivars. Although many microsclerotia were formed on subterranean plant parts, the contribution of these parts to the total production was only 3-17% in potato cv. Element and 8-44% in cv. Mirka. The number of microsclerotia per plant in the subterranean parts of 'Element' and 'Mirka' was similar for both cultivars. This implies that when haulm debris is removed from the field, much of the difference among potato cultivars in build-up of inoculum will disappear.

Crop debris is usually left in the field. When a good method to reduce microsclerotia formation in potato has been developed, attention should be paid to the reproduction of

V. dahliae in the other crops in the rotation too. Even the growth of wheat and barley may maintain a significant population in the soil, because of some reproduction of the fungus on the aerial and subterranean plant parts.

Isolate specificity

Isolate specificity to *V. dahliae* was proved to exist for both field bean and potato. Dry matter yield reductions of potato cv. Element and field bean were highest when these crops had been infected with their own isolates. In the field where one trial was performed the *V. dahliae* population seemed to be more pathogenic to potato than to field bean. Tjamos (1981) and Zilberstein et al. (1983b) found that the pathogenicity of *V. dahliae* depended on the cropping history of a field.

The finding that potato cultivars responded differently to potato and field bean isolate suggests that there may be differences in sensitivity among cultivars. Potato cvs Element, Ostara and Astarte were found to differ in the rate of build-up of the soil inoculum density, independent of the infestation level. However, potato cv. Mirka increased the population only at the high initial inoculum density in the soil, suggesting that the inoculum density has affects the plant's resistance.

Tuber yield of 'Element' was reduced when cropped after potato cvs Element and Astarte in the previous two years, but not when cropped after 'Ostara'. The yield after 'Mirka' was also reduced, whereas the build-up of the inoculum density in 'Mirka' was very small. From this it can be inferred that potato cultivars altered the population of microsclerotia, and the virulence of the microsclerotia formed differs among cultivars. This implies that in a rotation, the choice of the potato cultivar cropped may be an important factor to control both increase of soil infestation and yield loss.

Effect of haulm treatments on the population of Verticillium dahliae in soil

The results showed that the effect of a potato crop on the build-up of the soil infestation with *V. dahliae* can be considerably reduced in a crop rotation, if the formation of microsclerotia in aerial plant parts or the amount of aerial plant debris in the soil is reduced by cultural practices.

Haulm removal. Removing potato, flax and field bean debris led to a lower inoculum density in subsequent years and a slower increase of the inoculum density in continuous cropping with host crops.

The effect of removing the flax debris on soil inoculum density was significant after two crops. In potato, Easton et al. (1972) found that pre-harvest burning of haulm (similar to

removing in our experiment) only decreased the infestation level of heavily infested soil if the treatment was repeated for at least three years. Their results agree with our findings.

Harvest date. The number of microsclerotia formed on potato haulm depended greatly on the date of harvest. When the potato crop was harvested early in the growing season much fewer microsclerotia were formed than when the crop was harvested at maturity. *Verticillium dahliae* is a weak competitor for its food source (Ioannou et al., 1977a). At a more mature harvest, *V. dahliae* has already begun to grow to the outside of the tissue and the competing organisms will arrive too late to be able to prevent most of its reproduction. Moreover, the colonisation of the plant increases over time (Brandt et al., 1984).

Haulm treatments. A rapid desiccation of the haulm material will limit the formation of microsclerotia (Ioannou et al., 1977c; Erwin et al., 1978). Compared to killing with a herbicide, mechanical haulm killing reduced the formation of microsclerotia on potato debris. In contrast to chemical haulm killing or burning (using a propane heater), a cutting treatment leads to a sudden interruption in the water supply by the foliage. With the non-cutting treatments the time for the haulm to lose water will be longer than when the haulm is severed and left on the soil surface.

Implications. The two harvest dates used correspond with the moments the haulm is killed in The Netherlands for seed potatoes and ware potatoes. Given the findings of our experiments it can be assumed that the build-up of the population of microsclerotia in the soil will be much slower in fields where seed potatoes are grown than in fields cropped with ware potatoes.

For seed potatoes a mechanical haulm killing method together with incorporating the haulm into the soil can be recommended. At a late harvest date, the mechanical haulm treatment would be useful if the plant material desiccates in the field very quickly and will stay dry for some time, unless the haulm is completely mature at the time of the treatment. In The Netherlands, enough water will be available during senescence of the haulm to enable microsclerotia to form. In regions with a drier climate, mechanical haulm treatments might have a greater effect.

Theoretical approach to the dynamics of the inoculum density of Verticillium dahliae in the soil by a simple model

An equation based on biological and ecological principles and measurable parameters was developed to describe the inoculum densities of microsclerotia of *V. dahliae* in the soil. Fitting the mathematical equation to experimental data of potato ('Element', 'Ostara', 'Mirka' and

'Astarte'), flax, pea, barley, sugar beet, onion and field bean gave a very high correlation between observed and predicted soil inoculum densities. The results indicate that the model can adequately describe the population dynamics of *V. dahliae* in experimental field plots. The good correlation between observed and estimated values indicates that the assumptions made are compatible with the data obtained.

The values derived for a number of constants were consistent with values observed in other trials. With the derived constants, the model estimated the concentrations of microsclerotia in tissues of the various crops (including species immune to Verticillium wilt) which agreed closely with actual values measured in tissue of plants grown in moderately infested soil. This implies that not only a non-systemic infection of the root cortex, but also a low systemic infection may maintain a significant population of microsclerotia in the soil. At low initial inoculum densities, the inoculum build up for potato cv. Element is about twice as fast as that for the moderately resistant cv. Ostara and about 25 times faster than that for the highly resistant cvs Mirka and Astarte. The differences may diminish at higher inoculum densities due to the nature of the function for the formation of microsclerotia. At higher inoculum densities the more resistant cultivars will form more microsclerotia per m^2, because the concentration of microsclerotia increases much faster than the yield reduction.

The mortality rate estimated by the model results in the inoculum being reduced by 40% after one year and by 60% after two years. The shapes of the release and survival functions (Chapter 8: Fig. 1d) indicate that the population of microsclerotia in the soil is much more dynamic than is suggested by observations on soil inoculum densities over a long time span. Because of slow release and high persistence, a near steady state of the observed inoculum density is reached over a period of years.

Microsclerotia may continue to be released from the colonised potato debris for more than one year after crop debris has been incorporated into the soil (Chapters 5, 6 and 8). This agrees with the results of Ashworth et al. (1974) who buried infested cotton plant material in the soil. Since the mortality and release rates of microsclerotia are low, 'hardly-susceptible' crops should be grown continuously over many years to avoid yield loss by *V. dahliae*.

From the work done with the model it can be concluded that estimates of the development of the inoculum density as influenced by crop species and crop cultivars over a long time span could be useful for predicting the effects of *V. dahliae* on subsequent crops. Furthermore, it has been demonstrated that the model can be used to calculate the probable consequences of cropping sequences for the soil inoculum density.

Effect of the pathogen on crop yield

We did not find a close relation between soil inoculum density and crop yield. The observed tendencies of lower yields at high infestation levels and significant isolate effects indicate sufficient infection of crops in a field experiment (Chapter 5). However, all plants seemed to be infected at the end of the season at both infestation levels. Even the lower inoculum density was probably above the threshold that causes 100% infection in susceptible potato cultivars. The higher yields measured in soil with lower inoculum densities may have resulted from a later infection date (Nicot & Rouse, 1987). The greener potato haulm on plots with a lower inoculum density also suggested later infection and colonisation.

In Table 1 the dry matter yields of the experiment described in Chapter 7 are listed. In 1991 there was a large effect of soil infestation level on both the tuber yield of potato and the total aerial dry matter yield of field bean, because plants in the non-infested treatments suffered little or not al all from *V. dahliae*. However, at the end of the growing season microsclerotia had formed on the potato plants from non-infested plots too, indicating that these plants must have been infected during their growth.

In 1992, potato grown as a second crop after potato or after field bean showed lower yields in the initially infested treatments. No effect of the initial infestation was measured in barley and field bean after potato.

Table 1. Effect of soil infestation with microsclerotia in spring 1991 on the dry matter yields of potato (tubers), field bean (1991 total aerial parts; 1992 seeds) and barley (seeds) in 1991, 1992 and 1993.

Crop sequence			Dry matter yield (g.plot^{-1})								
1991	1992	1993	1991 Infested			1992 Infested			1993 Infested		
			No	Yes		No	Yes		No	Yes	
Potato[a]			642	358	***						
Potato[a]	Potato	Potato				535	479	***	325	419	***
Potato[a]	Barley	Potato				107	109	ns	429	456	ns
Potato[a]	Field bean	Potato				278	295	ns	360	407	**
Field bean[b]	Potato	Potato	279	199	***	656	575	***	360	426	**

*, ** and *** Infested differs significantly from not infested at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. ns = not significant.

[a]Infested plots infested with a potato isolate.

[b]Infested plots infested with a field bean isolate.

Soil analysis in the spring of the second year showed conclusively that no infestation had occurred in field bean in the field bean-potato sequence, whereas in the other initially non-infested plots *V. dahliae* was detected (Chapter 6: Table 1). Thus potato plants grown in plots that had been non-infested in the previous year and had carried a potato crop were easily infected by *V. dahliae*. This resulted in smaller yield differences for potato between originally non-infested and infested plots (Table 1). The absence of differences between originally non-infested and infested plots for yields of barley or field bean following potato year might be explained in the same way, but it is also very likely that barley and field bean were not sensitive to the potato isolate (Chapter 6).

In the infested plots, potato yields were higher after field bean than after potato ($P < 0.001$), even though the inoculum density in infested plots on the first sampling date was at least as high after field bean as after potato. This suggests that potato is less sensitive to the field bean isolate than to the potato isolate and confirms the results obtained in another experiment in the same field (Chapter 5).

In 1993, the potato-potato-potato, potato-field bean-potato, and field bean-potato-potato sequences showed similar tuber yields with a significantly higher yield on the initially infested soil (Table 1). Highest tuber yield was obtained from the potato-barley-potato sequence ($P < 0.05$), and for this sequence the infestation level had no significant effect. Easton et al. (1992) showed that higher potato tuber yields following cropping with non-hosts could be related to fewer *V. dahliae* propagules in the potato stems, even though the soil inoculum density had not decreased. Davis et al. (1994b) found that inoculum density did not decrease until after three years of cropping with a green manure crop, whereas the stem infection by *V. dahliae* decreased after one or two years. So, a factor besides the soil inoculum density must have affected the degree of stem infection of the plants. Wilhelm (1955) and Harrison (1976) reported achieving a substantial reduction of infection by *V. dahliae* after amending the soil with 0.4-1.6% barley straw, but the amount of debris of the crop in our experiment was much smaller. The more extensive root system and higher root mass of barley compared to potato and field bean may have caused differences in the soil microbiology and might explain why, in our experiments, potato tuber yields were higher after barley than after potato or field bean.

It is not clear why tuber yields were higher with higher inoculum densities in potato-field bean-potato and potato-potato-potato sequences. The composition of the population of microsclerotia in the soil may have played a role. Inoculum density increases faster in the initially non-infested treatments and therefore the population of microsclerotia in those treatments would have largely consisted of younger microsclerotia. Such microsclerotia will be more infective because more cells will produce hyphae when there is a stimulus to germinate

(Farley et al., 1971). Unfortunately, the detection method we used does not reveal the vitality of microsclerotia.

An alternative hypothesis is that antagonism to *V. dahliae* developed, triggering a decline. Gerlach (1968) found a strong decline of *Gaeumannomyces graminis* when wheat or barley was grown continuously compared to cropping these species in a 1:2 rotation. Van den Boogert and Velvis (1992) found a positive correlation between the increase of *Rhizoctonia solani* and of its mycoparasite *Verticillium biguttatum*. Scholte (Table 2) found a clear decline of black scurf (caused by *R. solani*) on tubers in pot experiments when potato was grown continuously, but in field experiments this decline was absent in clay soil and slight in sandy soil. With continuous cropping of potato no more black scurf occurred than in a 1/6 cropping frequency. The effect of antagonism was clearer in the pot experiment than in the field experiments. It seems probable that the development of antagonists was enhanced by the set-up of our container experiments. In containers like those we used in the field experiments, and in pots, the root/soil ratio will be higher than in a normal field situation and the roots may show an unnatural growth with a high density, especially along the walls of the containers. The combination of a higher inoculum

Table 2. Effect of cropping frequency with potato in sandy and clay soils on the incidence of black scurf (*R. solani*) on potato tubers.

Soil type	Cropping frequency of potato	Crop sequence last 3 years[b]	*Rhizoctonia solani* incidence[a] (black scurf index on tubers)	
			Pot experiment[c]	Field experiment[d]
Clay	1 / ≥ 4	o-o-p	23 c	17 b
	1 / 2	p-o-p	73 a	62 a
	1 / 1	o-p-p	37 b	-
	1 / 1	p-p-p	-	67 a
Sand	1 / ≥ 5	o-o-p	13 c	16 c
	1 / 2	p-o-p	56 a	65 a
	1 / 1	o-p-p	35 b	-
	1 / 1	p-p-p	15 c	55 b

[a]Different letters indicate significant differences between the treatments within an experiment at P=0.05 (according to the studentised range test of Tukey).

[b]Cropping sequence in the last three years; p=potato, o=other crop.

[c]Data obtained from K. Scholte (unpublished data).

[d]Data obtained from Scholte (1987).

density and a higher root density may have created an environment favouring the development of antagonists. If the antagonists are effective on the root surface, they will affect the infection of the roots, but not the soil inoculum density. This may be one of the factors causing a poor correlation between soil inoculum density and crop yield. From this it can be inferred that when conducting research to find antagonistic organisms it would be worthwhile investigating field soil used in pot experiments with intensive cropping of a host crop.

It is questionable whether the level of the inoculum density determines the disease severity in a very susceptible crop. As soon as *V. dahliae* was detectable in the soil, differences in potato tuber yield between the infestation levels decreased very rapidly, and differences in inoculum densitiy after removing the potato debris did not affect the yields. Easton et al. (1972) found that burning potato haulm enhanced tuber yield only if the initial soil inoculum density had been low. Although important, the composition of the soil inoculum and the source of the microsclerotia are not the only factors in the infection process.

General conclusions

- All crops stimulate the germination of microsclerotia of *V. dahliae* to some extent. Good hosts induce more microsclerotia to germinate than non-hosts. Potato cultivars also differ in their level of induction. However, none of the crop species or cultivars studied is able to decrease the soil inoculum density by inducting microsclerotia to germinate. Thus, *V. dahliae* cannot be controlled by growing 'lure' crops.
- Isolate specificity to *V. dahliae* has been demonstrated for field bean and for potato. A field bean isolate reproduced best on field bean and a potato isolate best on potato and flax.
- Microsclerotia had formed on the aerial debris of all crops investigated after they have been grown in infested soil. There are large differences in formation of microsclerotia among crop species and potato cultivars per g plant tissue and per m^2 soil surface. To control *V. dahliae*, attention should be paid to its potential maximum reproduction in all crops in a rotation. The formation of microsclerotia in most crops is sufficient to maintain a significant population in the field. However, given current farming practices, potato will contribute by far the most to the soil inoculum.
- Differences in the yield of microsclerotia among potato plant parts depend both on the density of microsclerotia and on the dry mass production per plant part. Although subterranean plant parts may contain considerable densities of microsclerotia, the contribution of the aerial parts is much larger for good hosts.
- In potato plants, microsclerotia formation is greatest on aerial stems and petioles.

- Removing potato, field bean or flax debris leads to a lower soil inoculum density in the subsequent years.
- On an early harvest date (seed potatoes) much fewer microsclerotia have formed on potato debris than on a late harvest date (ware potatoes).
- Compared to killing with a herbicide, mechanical haulm killing reduces the formation of microsclerotia on potato debris. A mechanical haulm killing method combined with incorporating the haulm into the soil can be recommended for seed potatoes. On a late harvest date, the mechanical haulm treatment is effective if the plant material desiccates in the field very quickly and remains dry for some time.
- Estimates of the development of the inoculum density as influenced by crop species and crop cultivars over a long time span can be helpful for predicting the effects of *V. dahliae* on subsequent crops. The model developed can be used to calculate the probable consequences of cropping sequences for the soil inoculum density.
- This thesis does not result in ready-to-use methods to control *V. dahliae*, but it has produced a useful analysis of the effect of cultural practices on the population dynamics. Moreover, this study provides a quantitative base on the interactions between crops and the fungus that deserves to be expanded in further research.

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SUMMARY

AGRONOMIC STUDIES ON THE POPULATION DYNAMICS OF *VERTICILLIUM DAHLIAE*

The pathogen

Diseases caused by *Verticillium dahliae* Kleb. are widespread throughout the world, wherever susceptible crops are grown, and are of economic importance in most countries. *V. dahliae* is the major component of the potato early dying complex that damages potato crops by causing wilt and early senescence of the crop. Hosts include all dicotyledonous plants, the most important crops affected being potato, cotton, egg-plant, tomato, mint, and olive.

Current efforts to reduce the use of nematicides to control potato cyst nematodes are expected to allow the population of other endoparasitic nematodes to increase. As *V. dahliae* shows a synergistic interaction with endoparasitic nematodes, it is also likely to increase and to cause more damage.

The pathogen survives as microsclerotia in soil. Their high survival potential and the wide host range mean that *V. dahliae* is endemic in many soils. Among field crops grown on arable farms in The Netherlands, potato is particularly severely affected by *V. dahliae*; yield losses are substantial and the fungus has a high reproduction. Therefore, in this study most attention was paid to potato. However, for a proper description of the population dynamics, the influence of all crops in a rotation on this pathogen must be considered.

Reducing the cropping frequency of potato is a possible method for controlling the soil population of *V. dahliae* and diminishing crop damage, but it is not attractive to Dutch farmers, because potato is a major cash crop in The Netherlands.

The quantitative information on the formation and mortality of microsclerotia of *V. dahliae* is poor and not very consistent. However, extensive literature is available on the life cycle and ecology of the fungus. It is reviewed in Section 1.2.

Effect of plant roots on the germination of microsclerotia

Laboratory experiments with root observation boxes were carried out to test the hypothesis that the induction of germination of microsclerotia by exudates from plant roots might be exploited as a measure to control *V. dahliae*. This involved testing the influence of seven crops on the germination of microsclerotia of *V. dahliae* in soil under controlled conditions (Chapter 2). The results of a field experiment in which the root density was measured were used to estimate the effect of crops on the population density of microsclerotia in the field (Section 2.2).

All crop species and cultivars investigated stimulated germination of microsclerotia more than a control without a crop. Host plants such as potato and field bean induced a higher percentage of the microsclerotia to germinate than a non-host such as barley. A susceptible potato cultivar stimulated germination more than a resistant cultivar. The germination percentage and the number of hyphae per microsclerotium decreased with distance from the root surface, regardless of the crop species or cultivar. Both the root zone affected and the root density were relevant for the effect on germination of microsclerotia in the soil. It is concluded that prospects are poor for growing a crop specifically to reduce the *V. dahliae* inoculum in the soil.

Formation of microsclerotia on crops

Image analysis for the quantification of microsclerotia of *V. dahliae* on plant material was compared with counting by eye (Chapter 3). The colonised plant material used was from the aerial and subterranean parts of potato plants grown in the greenhouse and of twelve crops grown in pots under natural conditions.

Image analysis proved to be a suitable and reliable method for assessing the number of microsclerotia in potato haulm samples from greenhouse experiments. The values obtained from colonised potato material from these experiments by image analysis were similar to those obtained by counting by eye. Most of the variation in the results could be attributed to sampling error. For most crops, image analysis overestimated the number of microsclerotia in plants grown outdoors, because plant and soil particles that did not discolour when the samples were boiled in sodium hydroxide.

The formation of microsclerotia on various crops was assessed (Chapter 4). This involved two pot experiments in which potato (cvs Element, Mirka, Ostara and Astarte), pea, sugar beet, onion, flax, spring barley, field bean, spring wheat and spring rape were infested with *V. dahliae* by root dipping or soil infestation (Section 4.1). In plants infested by root dipping, the dry matter yield and the formation of microsclerotia were determined for aerial parts only. In the pots with infested soil the dry weight and microsclerotial formation in stubbles and roots were also measured. In two greenhouse experiments, potato plants of the cvs Element and Mirka were artificially infested with *V. dahliae* (Section 4.2). Leaf blade, petiole, aerial stem, subterranean stem, stolon and root mass were harvested separately when the canopy was still green, and at maturity. After four weeks of incubation, plant tissue was air-dried and the numbers of microsclerotia per mg tissue and per plant were determined.

Within root dipping treatments, the most microsclerotia per g plant material and per pot were found in potato, flax and barley. In these treatments the microsclerotia density on potato

cv. Element, pea and barley was higher than in the soil infestation treatments, but the dry matter yields of the harvestable organs for potato cvs Element and Astarte, flax, sugar beet and barley were lower. In contrast, potato cvs Ostara and Mirka had fewer microsclerotia after root dipping than after soil infestation. The largest differences in the microsclerotial yield per pot among crops were found in the aerial parts. Flax gave the highest total microsclerotial yield per pot, followed by the four potato cultivars. The other crops yielded much fewer microsclerotia.

The highest numbers of microsclerotia in various plant parts were observed when potato cvs. Element and Mirka were harvested at maturity, rather than at an immature stage. By far the most microsclerotia were formed in the aerial plant parts. 'Element' yielded significantly more microsclerotia in the aerial plant parts than 'Mirka', but there were no differences between the two cultivars in the microsclerotial production on subterranean parts. The petiole and the aerial stem contributed most to the total microsclerotial production, whereas of the subterranean parts, roots contributed much more than stolons.

Effects of crop species, cultivars, and two isolates of Verticillium dahliae on the population of microsclerotia in the soil

Microsclerotia of *V. dahliae* form in large numbers on the senescing vegetative parts of host crops and remain viable in the soil for many years. Therefore it is difficult to control *V. dahliae* in crop rotations with a high incidence of host plants. In a micro-plot experiment the development of the inoculum density of *V. dahliae* in soil was studied and its consequences on yields of potato ('Element', 'Ostara', 'Astarte', and 'Mirka'), field bean, flax, pea, barley, sugar beet, and onion were evaluated (Chapter 5). For the experiment, 75-litre containers were filled with sterile soil in May 1991. The soil was then infested with 2 or 200 microsclerotia.g^{-1} of a potato isolate or a field bean isolate of *V. dahliae*. The same crop species and cultivar was grown in the same plot in 1991 and 1992. For flax, a non-pulled crop was compared with a pulled crop. Fallow plots were included as a control. In 1993, potato cv. Element was grown in all plots. The soil was sampled several times for three years, and the soil inoculum densities were assessed.

In the first year of the experiment (1991), yield differences between isolates were only significant in potato cv. Element (lower yield when infected with potato isolate), and potato cv. Astarte, field bean, and sugar beet (lower yield when infected with field bean isolate). In 1992, the isolates and infestation levels did not affect the yields. In 1993, the haulm dry matter yield of cv. Element was found to be influenced by the isolate, the inoculum density and the crop grown in the previous years. Plots previously cropped to potato cvs Element, Astarte and Mirka gave the lowest yields in 1993.

The inoculum densities of the fallow plots infested with only 2 microsclerotia.g^{-1} increased until the end of the third year, whereas the inoculum densities of the fallow plots infested with 200 microsclerotia.g^{-1} stabilised after the second year. Inoculum density did not decrease under any crop species or cultivar grown. The increase in inoculum density was significant in potato cvs Element, Ostara and Astarte, and field bean at the low initial soil infestation level. At the high initial soil infestation level the effect of crop species and cultivars on the inoculum density depended on the isolate used. Removing flax culms from the field considerably retarded the increase of the inoculum density in treatments infested with the potato isolate.

In 1993, the concentration of microsclerotia in the haulm debris correlated positively with the inoculum density in the soil, and the haulm yield correlated negatively with that density.

Effect of haulm treatments on the population of Verticillium dahliae in soil

The experimental factors in a second long-term micro-plot experiment consisted of two isolates of *V. dahliae*, one specific to field bean and one specific to potato, several crop sequences comprising potato, field bean and barley, and removal of aerial debris of the crops versus incorporation of debris into the soil (Chapter 6). Changes in the inoculum density were analysed over a period of three years.

The effect of potato haulm treatments was assessed in four pot experiments (Chapter 7). Potato plants of cv. Element were artificially infested with *V. dahliae*. On two harvest dates -one early and the other late- haulms were killed chemically, by burning, or by various other treatments, including cutting into pieces of different lengths and leaving the debris on the soil surface or covering it with soil. Four weeks later the plant material was air-dried and the number of microsclerotia per mg was determined.

It was found that potato was more susceptible to the potato isolate and field bean more susceptible to the field bean isolate. This implies that the pathogen tends to be host specific.

Removal of debris of potato and field bean diminished the inoculum density in the soil in subsequent years, but removal of barley straw had no effect. This is in accordance with the suitability of these crops as hosts: potato and field bean are good hosts and barley is a poor host. The mass of potato debris was inversely related to inoculum density in the soil. The control micro-plots that were initially non-infested subsequently became infested, probably because potato roots grew into the naturally infested subsoil. Inoculum density increased faster in the non-infested control micro-plots than in the initially infested treatments, because more colonised debris was produced. It is concluded that removing the aerial debris of host crops helps to reduce the density of *V. dahliae* inoculum in the soil.

In two of the four experiments, the chemical treatment yielded more microsclerotia on the early harvest date than the cutting treatments. Covering the colonised haulm tissue with non-sterilised soil was effective in inhibiting the microsclerotia formation. Shorter haulm pieces led to fewer microsclerotia on a late harvest date, when the material was left on the soil surface. Microsclerotial yield and treatment effects varied greatly among the different experiments.

Theoretical approach to the dynamics of the inoculum density of Verticillium dahliae in the soil by a simple model

In Chapter 8, a mathematical equation describing the inoculum densities of microsclerotia of *V. dahliae* in the soil over a long time span is developed. It is based on measurable parameters and ecological principles. In the model, the number of systemic infections of plant roots during crop growth are related to soil inoculum densities. In turn, the formation of microsclerotia in debris and reduction of the amount of the debris of different crops are related to the number of systemic infections. Finally, gradual release and mortality of microsclerotia in the soil are included, to enable subsequent inoculum densities in the soil to be calculated.

Fitting the mathematical equation to experimental data of potato ('Element', 'Ostara', 'Mirka' and 'Astarte'), flax, pea, barley, sugar beet, onion and field bean gave a very high correlation between observed and predicted soil inoculum densities. The clear differences in inoculum production among potato cultivars and other crops were expressed quantitatively. The highest inoculum density after incorporation of the debris of a susceptible crop was estimated to occur at 2.3 thermotime units of 3600 degree days (base 0 °C). Ten percent of the initial input of inoculum was still present after 4.5 thermotime units. The model was used to predict the dynamics of soil inoculum densities for *V. dahliae* under various cropping frequencies of potato.

Conclusions

1. Roots of crops stimulate germination of microsclerotia of *V. dahliae* to a certain but variable extent, nevertheless *V. dahliae* cannot be controlled in short crop rotations by growing a 'lure' crop.
2. Although there are large differences in the formation of microsclerotia among crop species and potato cultivars, microsclerotia formed on the aerial debris of all crops investigated.
3. Although subterranean parts of potato plants may yield considerable densities of microsclerotia, the contribution of the aerial parts is much larger.
4. On an immature harvest date much fewer microsclerotia will be formed on potato haulms than on a more mature harvest date.
5. Some isolate specificity to *V. dahliae* was proved for both field bean and potato.

6. Removing potato, field bean or flax debris from the field leads to a lower inoculum density in subsequent years, and retards the increase of the inoculum density in continuous cropping of the crop.
7. More microsclerotia formed on potato haulm killed by herbicide than on haulm killed mechanically.
8. The trends in the inoculum density over a long time span estimated by a model may be useful for predicting the effects of *V. dahliae* on subsequent crops and in crop rotations.

SAMENVATTING

AGRONOMISCH ONDERZOEK NAAR DE POPULATIEDYNAMIEK VAN *VERTICILLIUM DAHLIAE*

De ziekteverwekker

Ziektes veroorzaakt door *Verticillium dahliae* Kleb. komen wereldwijd voor op gronden waar gewassen worden geteeld die vatbaar zijn voor deze schimmel. De ziekte veroorzaakt in de meeste landen economische schade. *V. dahliae* is de belangrijkste van een groep pathogene organismen die verwelking van het gewas veroorzaken gevolgd door een vervroegde afsterving. Waardplanten zijn alle dicotylen, waarvan aardappel, katoen, aubergine, tomaat, munt en olijf de gewassen zijn die het sterkst worden aangetast.

V. dahliae laat een synergistische interactie zien met endoparasitaire nematoden. Door een vermindering van het gebruik van nematiciden, ter bestrijding van aardappelmoeheid, kan de dichtheid van endoparasitaire nematoden en daardoor de schade door *V. dahliae* toenemen.

De schimmel overleeft door het vormen van microsclerotia. De persistentie van microsclerotia en de brede waardplantenreeks maken V. *dahliae* tot een vaste bewoner van vele landbouwgronden. In de Nederlandse akkerbouw is aardappel een gewas waarin *V. dahliae* flinke opbrengstverliezen kan veroorzaken en waarin een hoge reproductie van de schimmel kan optreden. In dit onderzoek ligt dan ook de nadruk op dit gewas. Voor een goed inzicht in de populatiedynamiek moet echter de invloed van deze ziekteverwekker op alle gewassen in de rotatie in ogenschouw worden genomen.

Een lagere teeltfrequentie van aardappel kan één van de methoden zijn om de populatie van *V. dahliae* in de grond te beperken en om de schade in het gewas te verminderen. Dit is echter voor de akkerbouwer geen aantrekkelijke oplossing omdat aardappel een van de meest renderende gewassen in Nederland is.

Er is weinig kwantitatieve informatie over vorming en sterfte van microsclerotia van *V. dahliae*. Bovendien is deze informatie vaak niet eenduidig. Over de levenscyclus en ecologie van *V. dahliae* is wel veel literatuur beschikbaar. Een overzicht hiervan is gegeven in Hoofdstuk 1.2.

Invloed van plantewortels op de kieming van microsclerotia

Inductie van de kieming van microsclerotia door wortelexsudaten zou kunnen worden benut als een beheersingsstrategie van *V. dahliae*. De invloed van zeven gewassen op de kieming van microsclerotia is onderzocht onder gecontroleerde omstandigheden door middel van wortel-observatiekamers (Hoofdstuk 2). De resultaten van een veldproef, waarin de bewortelingsintensiteit werd gemeten, zijn gebruikt om een schatting te maken van de invloed van gewassen op de dichtheid van microsclerotia in het veld (Hoofdstuk 2.2).

Alle onderzochte gewassen stimuleerden de kieming van microsclerotia ten opzichte van een controle-object zonder gewas. Goede waardplanten, zoals aardappel, zetten een hoger percentage van de microsclerotia aan tot kieming dan een slechte waardplant, zoals gerst. Een vatbaar aardappelras stimuleerde de kieming meer dan een resistent ras. Het kiemingspercentage en het aantal hyfen per microsclerotium nam af met de afstand van het worteloppervlak, onafhankelijk van de gewassoort of het ras. Zowel de ruimte om de wortel die werd beïnvloed als de bewortelingsintensiteit waren belangrijk voor het totale effect op de populatie microsclerotia in de bodem. Geconcludeerd kan worden dat er weinig perspectieven zijn voor de teelt van een gewas om de inoculumdichtheid van *V. dahliae* in de bodem te verlagen.

Vorming van microsclerotia op gewassen

Beeldanalyse voor de kwantificering van microsclerotia van *V. dahliae* op plantmateriaal werd vergeleken met handmatig tellen (Hoofdstuk 3). Hiervoor werd gekoloniseerd plantmateriaal gebruikt van boven- en ondergrondse delen van aardappelplanten die waren geteeld in de kas en van twaalf gewassen die onder natuurlijke omstandigheden in potten werden geteeld.

Beeldanalyse bleek een geschikte en betrouwbare methode voor de bepaling van het aantal microsclerotia op monsters van aardappel die waren geteeld in de kas. De gemeten waarden in dit materiaal kwamen overeen met de resultaten van handtellingen. Spreiding in de resultaten werd hoofdzakelijk veroorzaakt door de monstername. Voor de meeste gewassen die buiten werden opgekweekt werd het aantal microsclerotia in het plantmateriaal overschat bij gebruik van beeldanalyse. Deze overschatting werd veroorzaakt door plante- en bodemdeeltjes die niet ontkleurden tijdens de voorbehandeling die bestond uit het koken met natronloog.

De vorming van microsclerotia op verschillende gewassen werd bepaald (Hoofdstuk 4). In twee potproeven werden de gewassen aardappel (rassen Element, Mirka, Ostara en Astarte), erwt, suikerbiet, ui, vlas, zomergerst, veldboon, zomertarwe en zomerkoolzaad besmet door inoculatie van de wortel of door besmetting van de grond (Hoofdstuk 4.1). Van planten waarvan de wortels werden geïnoculeerd werd het aantal microsclerotia en de opbrengst alleen in de bovengrondse delen bepaald. Van de planten in de potten waarvan de grond was besmet,

werden ook de opbrengst en het aantal microsclerotia in de stoppel en de wortel bepaald. In twee kasproeven werden aardappelplanten van de rassen Element en Mirka kunstmatig besmet met *V. dahliae* (Hoofdstuk 4.2). Bladschijf, bladsteel, bovengrondse stengel, ondergrondse stengel, stolon en wortel werden gescheiden geoogst op een tijdstip dat het gewas nog groen was en bij een afgerijpt gewas. Na een incubatietijd van vier weken werd het plantmateriaal luchtdroog gemaakt en de aantallen microsclerotia per mg plantmateriaal en per plant werden bepaald.

Binnen de behandelingen waarbij de wortels van de planten waren geïnoculeerd werden de hoogste aantallen microsclerotia per mg en per plant gevonden in aardappel, vlas en gerst. De dichtheid van de microsclerotia op aardappelras Element, erwt en gerst was hoger, terwijl de drogestof opbrengsten van de oogstbare delen van de aardappelrassen Element en Astarte, vlas, suikerbiet en gerst lager waren bij inoculatie van de wortel dan bij besmetting van de grond. De aardappelrassen Ostara en Mirka daarentegen vormden minder microsclerotia bij inoculatie van de wortel dan bij besmetting van de grond. De grootste verschillen in de aantallen microsclerotia werden gevonden in de bovengrondse delen. Vlas vormde het hoogste totaal aantal microsclerotia per pot, gevolgd door de vier aardappelrassen. De andere gewassen vormden veel minder microsclerotia.

Vergeleken met de oogst van nog groene planten werden meer microsclerotia op de verschillende delen van de planten van de aardappelrassen Element en Mirka gevonden bij de oogst van een rijp gewas. Verreweg de meeste microsclerotia werden gevormd op de bovengrondse delen. Op de bovengrondse delen was het aantal microsclerotia op 'Element' significant hoger dan op 'Mirka', terwijl er geen verschil optrad op de ondergrondse delen. De bladstelen en de bovengrondse stengel droegen het meeste bij aan de totale aantallen. De wortels droegen meer bij aan de ondergrondse produktie van microsclerotia dan de stolonen.

Invloed van gewassen, rassen en twee Verticillium dahliae isolaten op de populatie microsclerotia in de bodem

Microsclerotia van *V. dahliae* worden in grote aantallen gevormd op afstervende vegetatieve delen van gewassen, waarna ze vele jaren in de grond kunnen overleven. Het is dan ook moeilijk om *V. dahliae* te beheersen in gewasrotaties met een groot aandeel waardplanten. Het verloop van de inoculumdichtheid in de grond en de gevolgen voor de opbrengst van aardappel ('Element', 'Ostara', 'Mirka' en 'Astarte'), veldboon, vlas, erwt, gerst, suikerbiet en ui werden onderzocht in een micro-plot experiment. In mei 1991 werden 75 l grote containers gevuld met gesteriliseerde grond. Deze grond werd besmet met 2 of 200 microsclerotia per g van een aardappel- of een veldboonisolaat van *V. dahliae*. In 1991 en 1992 werd hetzelfde gewas

verbouwd op elk veldje. Getrokken vlas werd vergeleken met niet getrokken vlas. Braak liggende veldjes werden aangelegd als controle. In 1993 werd op alle veldjes het aardappelras Element verbouwd. Gedurende drie jaar werd de grond bemonsterd en werd de inoculumdichtheid bepaald.

In het eerste jaar (1991) reageerde het aardappelras Element met een lagere gewasopbrengst bij besmetting met het aardappelisolaat, terwijl aardappelras Astarte, veldboon en suikerbiet juist een lagere gewasopbrengst gaven bij besmetting met het veldboonisolaat. In 1992 werd geen effect van isolaat en besmettingsniveau op de opbrengst aangetoond. In 1993 werd de loofopbrengst van aardappelras Element beïnvloed door het isolaat, de inoculumdichtheid en de voorvrucht. De behandelingen met als voorvruchten de aardappelrassen Element, Astarte en Mirka gaven de laagste opbrengsten.

In de licht besmette controle-veldjes stegen de inoculumdichtheden tot aan het einde van het derde jaar, terwijl de inoculumdichtheden in de zwaar besmette controle veldjes zich stabiliseerden na het tweede jaar. Onder geen van de gewassen werd een daling van de inoculumdichtheid aangetoond. Bij het lage besmettingsniveau steeg bij de aardappelrassen Element, Ostara en Astarte en voor veldboon de inoculumdichtheid significant. Bij het hoge besmettingsniveau was de invloed van het gewas op de inoculumdichtheid afhankelijk van het gebruikte isolaat. Het verwijderen van de stengels van het vlas vertraagde de stijging van de inoculumdichtheid aanzienlijk in behandelingen besmet met het aardappelisolaat.

In 1993 vertoonde de concentratie van de microsclerotia in de stengelresten een positieve correlatie met de inoculumdichtheid in de grond en de stengelopbrengst vertoonde een negatieve correlatie met de inoculumdichtheid in de grond.

Invloed van loofbehandelingen op de Verticillium dahliae populatie in de grond

In een tweede meerjarige veldproef met micro-plots werden twee *V. dahliae* isolaten (één specifiek voor veldboon en één specifiek voor aardappel), verschillende vruchtopvolgingen van aardappel, veldboon en gerst en verwijdering van oogstresten als factoren opgenomen (Hoofdstuk 6). Het verloop van de inoculumdichtheid in de grond werd gevolgd over een periode van drie jaar.

De invloed van loofbehandelingen van aardappel werd onderzocht in vier potproeven (Hoofdstuk 7). Planten van het aardappelras Element werden geïnoculeerd met *V. dahliae*. Het aardappelloof werd gedood door behandeling met een herbicide, door branden, of door verschillende andere behandelingen, waaronder het snijden in stukken van verschillende lengtes en het plaatsen van de stukjes op het grondoppervlak of het bedekken van de stukjes met grond. Na

vier weken werd het plantmateriaal luchtdroog gemaakt en werd het aantal microsclerotia bepaald.

Waardplantspecificiteit kon worden aangetoond. Aardappel was vatbaarder voor het aardappelisolaat en veldboon vatbaarder voor het veldboonisolaat.

Verwijdering van de oogstresten van aardappel en veldboon zorgde voor een afname van de inoculumdichtheid in de grond in de volgende jaren, maar de verwijdering van gerstestro had geen invloed. Dit komt overeen met de waardplantgeschiktheid van deze gewassen: aardappel en veldboon zijn goede waardplanten en gerst is een slechte waardplant. De hoeveelheid oogstresten was kleiner bij een hogere initiële inoculumdichtheid in de grond. De oorspronkelijk niet besmette controleveldjes raakten toch besmet. Dit gebeurde waarschijnlijk door de groei van aardappelwortels vanuit de containers naar de ondergrond. Omdat in de niet besmette controleveldjes een grotere hoeveelheid gekoloniseerde oogstresten werd geproduceerd steeg de inoculumdichtheid daar sneller dan de inoculumdichtheid in de besmette objecten. Verwijdering van gewasresten van waardplanten bleek een effectieve methode om de inoculumdichtheid van *V. dahliae* in de bodem te verlagen.

In twee van de vier proeven leidde bij oogst op een vroeg tijdstip een chemische loofbehandeling tot vorming van meer microsclerotia dan de mechanische loofbehandelingen. Door het stengelmateriaal met grond te bedekken kon de vorming van microsclerotia effectief worden geremd. Het verkorten van de stengelstukjes leidde tot een lagere produktie van microsclerotia wanneer het materiaal op het grondoppervlak werd bewaard. De variatie in aantallen microsclerotia en in de behandelingseffecten tussen de verschillende proeven was groot.

Theoretische benadering van de dynamiek van de inoculumdichtheid van Verticillium dahliae in de grond door middel van een eenvoudig model

In Hoofdstuk 8 is een rekenkundige vergelijking opgesteld die het verloop van de inoculumdichtheid van microsclerotia van *V. dahliae* in de bodem beschrijft over lange tijd. De vergelijking werd gebaseerd op meetbare parameters en principes met een ecologische betekenis. In het model werd het aantal systemische infecties van de plantewortels gedurende de groei van het gewas gerelateerd aan de inoculumdichtheid in de bodem. Vervolgens werd de vorming van microsclerotia in oogstresten en de opbrengst van oogstresten gekoppeld aan het aantal systemische infecties. In de laatste fase werden een geleidelijk vrijkomen en een geleidelijke afsterving van microsclerotia opgenomen om toekomstige inoculumdichtheden in de grond te berekenen.

Het toetsen van de rekenkundige vergelijking met experimentele gegevens van aardappel (rassen Element, Ostara, Mirka en Astarte), vlas, erwt, gerst, suikerbiet, ui en veldboon resulteerde in een zeer hoge correlatie tussen waargenomen en berekende inoculumdichtheden.

De duidelijke verschillen tussen aardappelrassen en andere gewassen werden gekwantificeerd. De hoogste inoculumdichtheid na het in de grond brengen van oogstresten werd geschat op 2.3 temperatuursom eenheden van 3600 graaddagen (basis 0 °C). Tien procent van het inoculum was nog aanwezig na 4.5 temperatuursom eenheden. Het model werd gebruikt om voorspellingen te doen omtrent de dynamiek van inoculumdichtheden van *V. dahliae* in de bodem onder verschillende teeltfrequenties van aardappel.

Conclusies

1. Gewaswortels hebben een stimulerende invloed op de kieming van microsclerotia van *V. dahliae*, waarbij goede waardplanten meer stimuleren dan slechte waardplanten en vatbare aardappelrassen meer dan resistente rassen. Desondanks kan in gewasrotaties *V. dahliae* niet worden beheerst door het telen van een 'lokgewas'.
2. Microsclerotia kunnen worden gevormd op of in bovengrondse delen van de meeste gewassen, ook in die van monocotyle gewassen zoals gerst en tarwe. Er bestaan echter grote verschillen tussen gewassen in de mate van vorming van microsclerotia.
3. Hoewel in ondergrondse delen van aardappelplanten aanzienlijke dichtheden van microsclerotia kunnen worden gevormd, is de vorming op de bovengrondse delen aanzienlijk groter.
4. Na de loofdoding van aardappel op een tijdstip waarop het loof nog groen en onrijp is worden veel minder microsclerotia gevormd dan na loofdoding op een tijdstip waarop het loof rijp is.
5. Isolaatspecificiteit is aangetoond voor veldboon en aardappel.
6. Verwijdering van het veld van oogstresten van aardappel, veldboon of vlas leidt tot een lagere inoculumdichtheid in de bodem in de daaropvolgende jaren.
7. Vorming van microsclerotia is veel lager bij toepassing van mechanische loofdodingstechnieken dan bij loofdoding met een chemisch middel.
8. Schatting van de inoculumdichtheid over een lange tijdsperiode door middel van een model is een geschikte methode om de invloed van *V. dahliae* op gewassen en in gewasrotaties te voorspellen.

CURRICULUM VITAE

Leendert Mol werd geboren op 30 maart 1965 te Kattendijke. Na het behalen van het VWO-diploma aan het Christelijk Lyceum voor Zeeland te Goes begon hij in 1983 met de studie Landbouwplantenteelt aan de toenmalige Landbouwhogeschool te Wageningen. In september 1988 behaalde hij het doctoraalexamen met als afstudeervakken Akkerbouw, Bodemkunde en plantevoeding en Produktkunde. Door het volgen van de Eénjarige Lerarenopleiding voor Afgestudeerden van de LUW bij STOAS te Wageningen van augustus 1988 tot juli 1989 verkreeg hij de onderwijsbevoegdheid. In januari 1990 werd hij voor één jaar aangesteld als toegevoegd docent bij de toenmalige vakgroep Landbouwplantenteelt en graslandkunde met als belangrijkste taak het schrijven van de collegedictaten Zaaizaad- en Pootgoedproduktie, Beschrijvende Landbouwplantenteelt en Geïntegreerde Plantaardige Produktie. Per 1 januari 1991 trad hij in dienst bij de eerder genoemde vakgroep als assistent in opleiding en verrichtte het onderzoek dat beschreven is in dit proefschrift in het kader van het additioneel onderzoeksprogramma behorende bij het Meerjarenplan Gewasbescherming.